

NEW METHODS FOR THE SYNTHESIS OF
HIGHER-CARBON AMINO-DEOXY-SUGARS.
SYNTHESIS OF N-ACETYLNEURAMINIC ACID
AND SOME OF ITS ANALOGUES

Inaugural-Dissertation

Submitted for the
Degree of Doctor of Philosophy
to the Philosophical Faculty II
of the
University of Zürich

by
FRANZ BAUMBERGER
of Balterswil (TG)

Approved by Prof. Dr. A. Vasella

Zürich 1987
Zentralstelle der Studentenschaft

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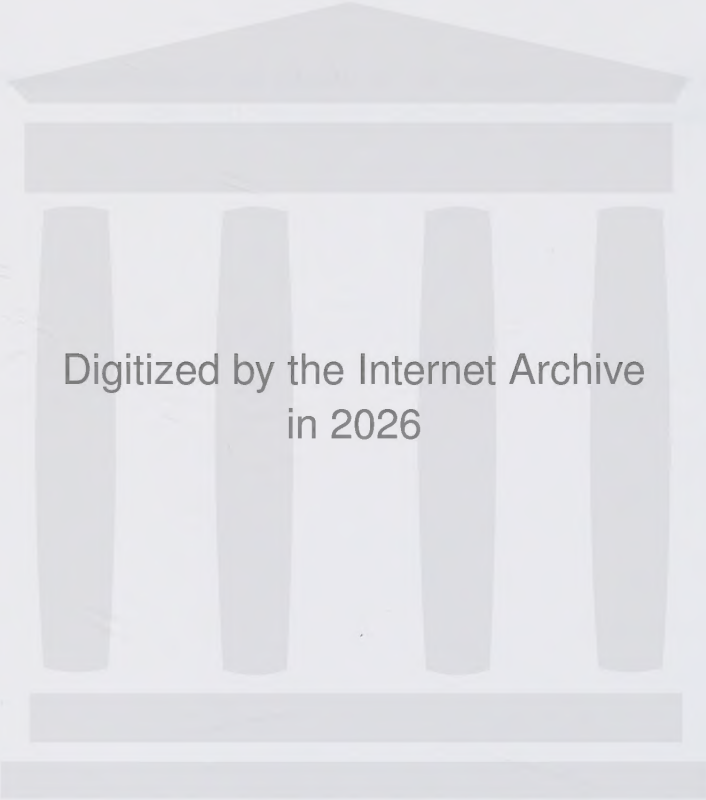
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To Isabella

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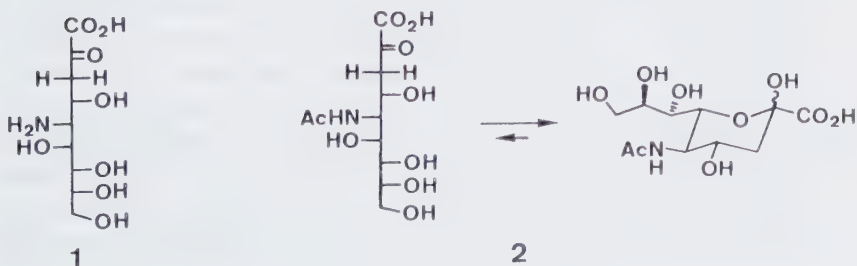
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THEORETICAL PART

1. Introduction

1.1. History of N-Acetylneuraminic Acid

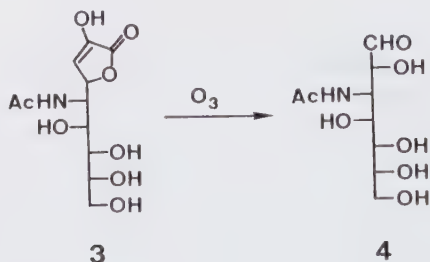
Recognition of the importance of sialic acids for the regulation of a great variety of biological phenomenas has fueled the interest in these compounds over the last years. The name 'sialic acid' was proposed in 1957 by Blix, Gottschalk and Klenk [1] for the N- and/or O-acylated derivatives of neuraminic acid **1** [2]. The term 'neuraminic acid' was first used by Klenk [3] for a compound isolated after acidic methanolysis of brain gangliosides and later identified [4] as methyl β -D-glycoside of N-acetylneuraminic acid.



N-acetylneuraminic acid (Neu5Ac, **2**) was first isolated by Gottschalk [5] from ovomucoid and urinary mucins, which were exposed to influenza viruses. Although the functional groups of N-acetylneuraminic acid (**2**) have

been known early, it took a long time to establish the whole structure of **2**. The synthesis of Neu5Ac **2** from N-acetyl-D-glucosamine and oxaloacetate [6] (see Chap. 1.5.2), and the enzymatic degradation of Neu5Ac (from natural sources) [7] have proven the configuration at C(5) - C(8). The configuration at C(4) was determined by ozonolysis of the γ -lactone **3** giving the 3-acetamido-3-deoxy-D-glycero-D-galacto-heptose (**4**; Scheme 1), identical (m.p., $[\alpha]_D$) with an authentic sample [8].

Scheme 1



The progress in analytical methods over the last 30 years led to the detection and isolation of more than twenty O- and/or N-acyl derivatives of neuraminic acid, the most important being N-acetylneuraminic acid **2**, its conformation was determined by 1H -NMR-spectroscopy [9]. N-acetylneuraminic acid (**2**) exists in the 2C_5 (2,6)-pyranose form, which is stabilized by intramolecular hydrogen bonds [10]. In H_2O , **2** exists as a 8:92 mixture of the α - and β -D-anomers, respectively, the major one possessing an axial anomeric OH-group. The structure of the β -D-anomer has been confirmed by X-ray analysis [11][12]. The naturally occurring glycosides of **2**, however, are α -D-configured (with the exception of CMP-Neu5Ac, see Chap. 1.4).

1.2. Occurrence of Sialic Acids

Sialic acids have been found in animal tissues and in some viruses and bacteria, but not in plants (for reviews see [13-15]). Viruses known to possess a sialidase as part of their membrane, e.g. some strains of myxoviruses or influenza viruses, do not contain sialic acid [16]. Bacteria possessing sialic acids are e.g. *E. coli*, *N. meningitidis* and *Salmonella* strains. All of them are hosted by mammals and some are even pathogenic. In lower animals, sialic acids have apparently not been found [17], but they generally occur in vertebrates [13][15]. The greatest variety of sialic acids has been found in mammals, where they have been detected for example in:

- a) oligosaccharides of milk and colostrum [18-20]
- b) the plasma proteins and (to small extent) in the serum
- c) most of the mucus glycoproteins
- d) membrane glycoproteins
- e) hormones and enzymatically active glycoproteins (e.g. transferases and hydrolases)
- f) glycolipids (e.g. gangliosides).

In the last years two correlations were detected: the later an animal form appears, the less O-acyl sialic acids it contains and, while in most animal tissues mixtures of N-acetyl- and N-glycolylneuraminic acid are found, in human tissues only Neu5Ac occurs.

Sialic acids are usually found in low concentrations as free molecules in biological materials (1 - 50 μmol)¹⁾. They are mostly components of oligosaccharides and glycoconjugates [18][22], where the sialic acid moieties occupy the nonreducing end. In gangliosides, they may also be linked in side positions of the oligosaccharide chain. Sialic acid residues are preferentially $\alpha(2,3)$ bound to galactose [23][24], $\alpha(2,6)$ to N-acetylgalactosamine [25] or $\alpha(2,8)$ to another N-acetylneuraminic acid residue [26].

¹⁾ 98% of orally administered ^{14}C -labeled Neu5Ac is excreted in the urine within 6 h; in 90% i.v. administered ^{14}C -labeled Neu5Ac is excreted within 10 min [21].

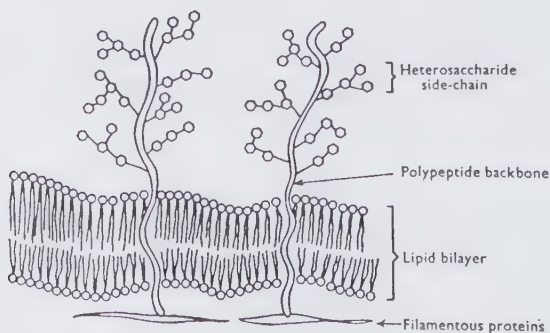
1.3. Biological Significance of Sialic Acids

Many functions have been attributed to the glycosidically linked sialic acid residues on oligosaccharide chains of glycolipids and glycoproteins. Some have been proven, others are still hypothetical, but the important role they play in biological processes is evident (for reviews see [14] and [27]). The following examples are intended to illustrate this point.

1.3.1. Neu5Ac containing Glycoproteins and Glycolipids in the outer Membrane of Erythrocytes

At physiological pH and ionic strength, the erythrocytes from many animals exhibit a negative electrophoretic mobility [28], denoting their negative surface charge. Hanig [29] and Ada and Stone [30] have shown that influenza viruses and also sialidase of *Vibrio cholerae* greatly reduce the erythrocyte mobility. Klenk and Uhlenbruck [31] have shown that sialidase acts on the erythrocyte stroma by releasing Neu5Ac, which they crystallized. Hence, N-acetylneuraminic acid 2 must be determining for the negative surface charge [32]. It is said that this negative surface charge protects the cells against outside attacks [33] and that it prevents their aggregation by electrostatic repulsion. Apart from the electrostatic repulsion, the sialic acid moieties in Glycophorin A (a membrane glycoprotein of erythrocytes with a high content of Neu5Ac, see Fig. 1) seem to mask the D-galactose residues, to which they are linked [34].

Figure 1: Major glycoprotein of the human erythrocyte membrane (schematic, [33]).

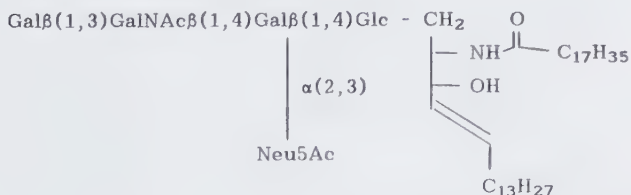


Treatment of erythrocytes in vitro with sialidase or i.v. administration of this enzyme led to a drastic decrease of the erythrocyte life-span [35]. Asialo erythrocytes are rapidly agglutinated in the liver. This accumulation is a function of a lectinlike receptor on the 'Kupfer' cells, which recognizes the unmasked D-galactose residue on the asialo erythrocytes. The agglutination can be strongly inhibited by addition of N-acetylgalactosamine, while other sugars are mostly without any effect [34][36]. This masking effect of Neu5Ac can be reconstituted by treating sialidase-treated erythrocytes with Neu5Ac-CMP and sialyltransferases [37].

1.3.2. Sialic Acids and Gangliosides

Gangliosides, e.g. G_M1 (see Fig. 2) are a group of glycosphingolipids containing one or more sialic acid residues [38][39]. Many biological functions have been attributed to the sialic acid residues on gangliosides. Some of these are still hypothetical, others have been proven.

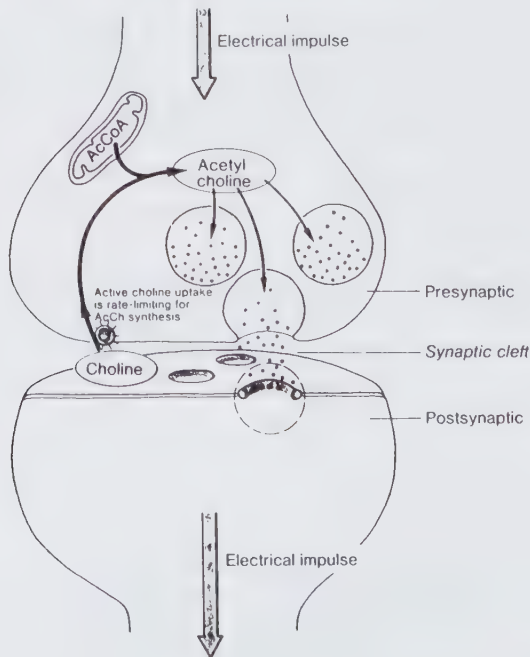
Figure 2: Ganglioside G_M1



Although gangliosides are present in most mammalian tissues, they are concentrated in the plasma membrane of nerve cells, especially in the region of nerve endings and dendrites [40]. There is good evidence that gangliosides are involved in the release of neurotransmitters [41][42]. Gangliosides possess a strong and a weak binding site for Ca^{2+} . Both the glycerol side chain and the carboxylate group of the Neu5Ac residues are thought to be responsible for the Neu5Ac-Ca interaction [43][44].

In 1980, Svennerholm [42] proposed a model for the synaptic transmission (see Fig. 3). The role of gangliosides at the synaptic junctions is to bind Ca^{2+} , which is released at each impulse and transported in specific Ca^{2+} channels into the presynapse. The removal of Ca^{2+} from the gangliosides (at each impulse) should lead to an unstability of the presynaptical membrane and facilitate a fusion with the membrane of the synaptic vesicles. The increased concentration of Ca^{2+} within the presynapse will result in the contraction of the microfilaments leading to the ejection of the neurotransmitter into the synaptic cleft. The increased concentration of Ca^{2+} within the presynapse will result in the contraction of the microfilaments leading to the ejection of the neurotransmitter into the synaptic cleft.

Figure 3: Cholinergic neurotransmission (schematic, [45]).



The Neu5Ac residues of the gangliosides are also said to participate in the uptake of the positively charged transmitter (e.g. Acetylcholine). Since the Neu5Ac moieties of gangliosides on the presynapse possess Na^+ as

counter ion are more strongly dissociated than those of the postsynapse, which are present as Ca^{2+} salts, there exists a charge difference that will cause the backmoving of the (positively charged) transmitter to the presynapse.

Membrane gangliosides may also play an important role as receptors for different toxins [46-49]. It has been known for some time that influenza viruses bind to cells by interacting with membrane receptor molecules containing sialic acid residues and that the virus component involved is the haemagglutinin [50]. Comparative analysis of the haemagglutinins of different viruses have demonstrated preferences for binding to oligosaccharide chains containing Neu5Ac residues in either $\alpha(2,6)$ or $\alpha(2,3)$ -linkages to the penultimate residue. For example, the haemagglutinin of the 1968 Hong Kong influenza virus recognizes sialyloligosaccharides with the terminal sequence Neu5Ac- $\alpha(2,6)$ -Gal [50]

Furthermore, sialic acid containing compounds play a role in:

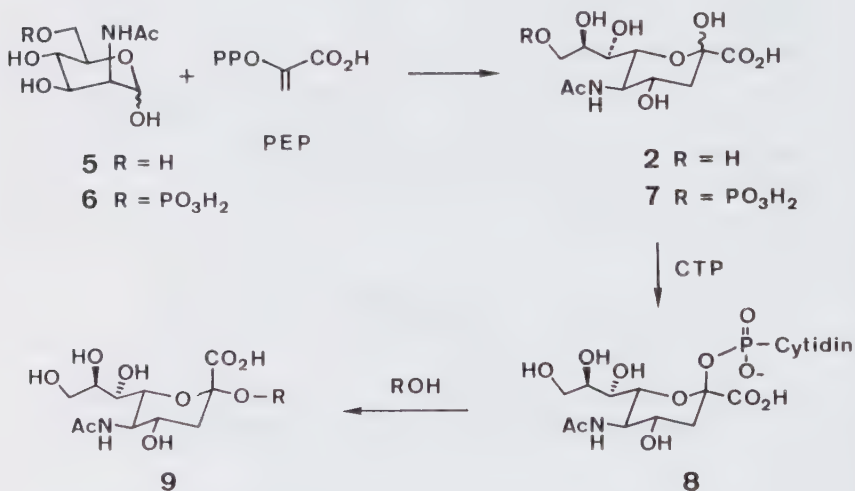
- the viscosity of mucins [51]
- blood coagulation [52]
- cell recognition and cell adhesion [53]
- immunological processes and infectious diseases [54].

1.4. Biosynthesis of Neu5Ac and Enzymatic Glycosidation of Neu5Ac

The biosynthesis of N-acetylneuraminic acid has been elucidated by feeding radioactive monosaccharides as precursors (e.g. N-acetylmannosamine, which is a specific marker for sialic acid [55]) and by isolation and purification of specific enzymes [56]. Two enzymes have been found to catalyze the synthesis of Neu5Ac 'in vivo'. Neu5Ac-synthase (EC 4.1.3.19) from *N. meningitidis* [57] catalyzes the reaction of N-acetylmannosamine (ManNAc, 5) with phosphoenolpyruvate (PEP) giving Neu5Ac 2 (Scheme 2). This pathway is used by bacteria, e.g. *N. meningitidis*. The biosynthesis of Neu5Ac in mammals requires the action of a specific kinase (EC 2.7.1.60) for phosphorylation of the C(6) OH group of ManNAc 5 giving 6 [58]. Neu5Ac-9-P synthase (EC 4.1.3.20) [58][59] then catalyzes the reaction of Man-

NAc-6-phosphate **6** with PEP to give N-acetylneuraminic acid 6-phosphate **7**. After condensation, the C(9) phosphate group in **7** is removed. A specific Neu5Ac-9-P phosphatase (EC 3.1.3.29) has been isolated from human erythrocytes [60], but dephosphorylation occurs also in the presence of a nonspecific phosphatase [58].

Scheme 2



Two enzymes are involved in the formation of Neu5Ac glycosides. A cytidyltransferase (found in the nucleus of liver, spleen, kidney and brain cells [61]) catalyzes the reaction of Neu5Ac **2** with Cytidintriphosphate (CTP) to CMP-Neu5Ac **8** and pyrophosphate (Scheme 2). The 'activated Neu5Ac' **8** is the only known naturally occurring glycoside of Neu5Ac pos-

- 2) Acylneuraminate pyruvate-lyase [8b] also catalyzes the reaction of ManNAc **5** with pyruvate (see chap. 1.5.4). Although this enzyme is widely distributed in bacteria and mammalian tissues, no sialic acids were found in those bacteria and the enzyme is absent from tissues that produce large quantities of sialic acids in mammalia.

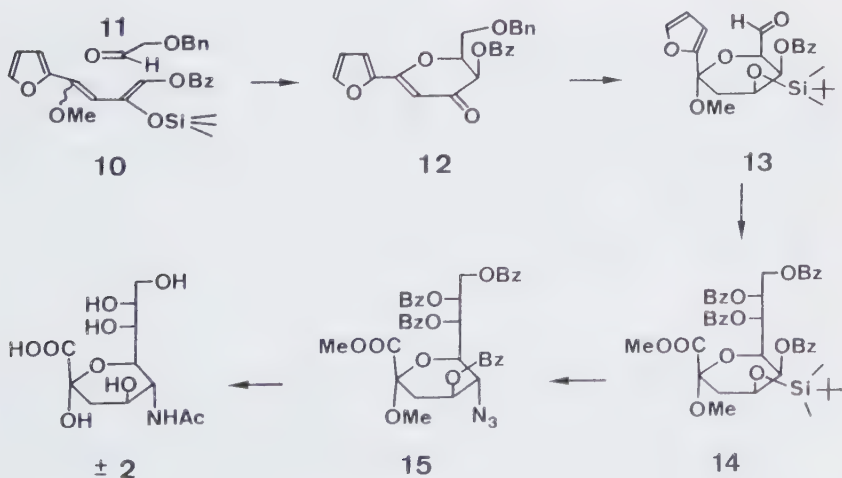
sessing the β -D-configuration [62]. Specific sialyltransferases (differing mainly in their acceptor specificity) are required for the transfer of Neu5Ac residues from CMP-Neu5Ac **8** to the penultimate sugar of the acceptor molecule, giving an α -D-configured glycoside **9** [55][63]. This transformation seems to follow a (partial) S_N -2-type mechanism, since it involves inversion of configuration at C(2) of the Neu5Ac residue.

1.5. Syntheses of Sialic Acids

1.5.1. Synthesis of Neu5Ac from Nonsugar Compounds

The first synthesis of D,L-Neu5Ac starting from nonsugar compounds has been described by Danishefsky and De Ninno in 1986 [64][65]. A first key step in this synthesis is the Lewis acid-catalyzed cycloaddition of the oxygenated diene **10** to 2-benzyloxyacetaldehyde (**11**) to give the pyrone **12**

Scheme 3



(Scheme 3)³). Stereoselective reduction of the carbonyl group in 12, followed by the addition of MeOH to the enol ether group, protection of the C(4) hydroxy group, hydrogenolysis of the benzyloxy group and finally oxidation of the primary hydroxy group at C(7) gave the aldehyde 13. Chain elongation at C(7) to a Z-olefinic ester (with Emmons reagent), stereoselective bishydroxylation (OsO₄) of the C,C double bond, reduction of the aliphatic ester function followed by benzoylation of the triol and oxidative degradation of the furane ring with subsequent esterification gave the methylester 14. Desilylation of 14 (HF, MeOH) was followed by an O→O benzoyl migration. Mesylation of the C(5) hydroxy group and displacement by NaN₃ yielded the azide 15. Reduction of the azido function and acetylation produced a fully protected methylglycoside of N-acetylneuraminic acid, which upon saponification and acidic workup (Dowex 50) gave the racemic N-acetylneuraminic acid (rac-2) in an overall yield of less than 9% (the yields of 6 steps are not indicated).

1.5.2. Syntheses of Sialic Acids from Sugars

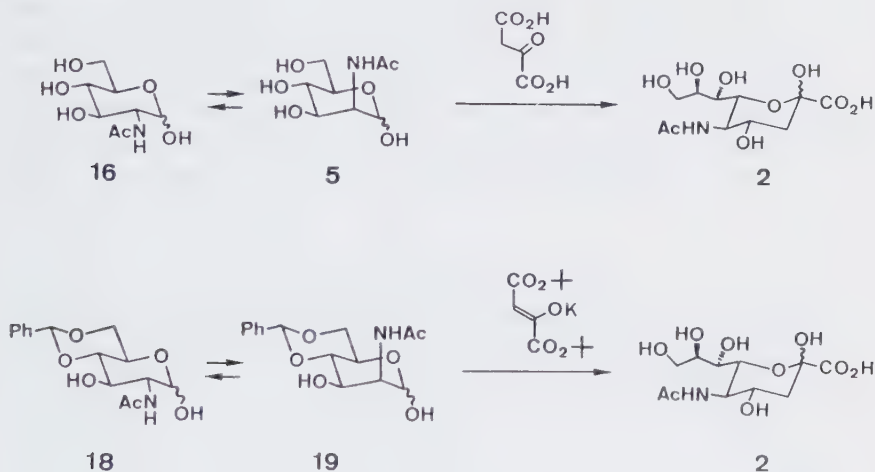
In alkaline solutions (pH 11) N-acetylglucosamine (GlcNAc, 16) equilibrates with N-acetylmannosamine (ManNAc, 5) [68][69]. In the first synthesis of N-acetylneuraminic acid 2 by Cornforth et al. [6], the mixture of 5 and 16 was treated with oxaloacetic acid 17⁴) at pH 11 to give, after decarboxylation, Neu5Ac 2 in a yield of 1-2% (Scheme 4). This yield was later improved to ca. 10% by using pure ManNAc 5 in the aldol reaction.

³) The diene 10 was prepared in 3 steps (76%) from acetylfurane [66]. The aldehyde 11 was synthesized from 2-propenol by benzylation of the hydroxy group and ozonolysis of the C,C double bond in a yield of 80% [64][67].

⁴) Oxaloacetic acid 17 is now commercially available. Before, it has been enzymatically prepared from L-malic acid with Malate dehydrogenase [70]

How and coworkers [71] modified this synthesis using sodium tetraborate in the epimerisation step [GlcNAc $\rightleftharpoons$ ManNAc] to stabilize the configuration of N-acetylmannosamine **5**. Their yield of Neu5Ac (on a preparative scale) amounted to 21%.

Scheme 4



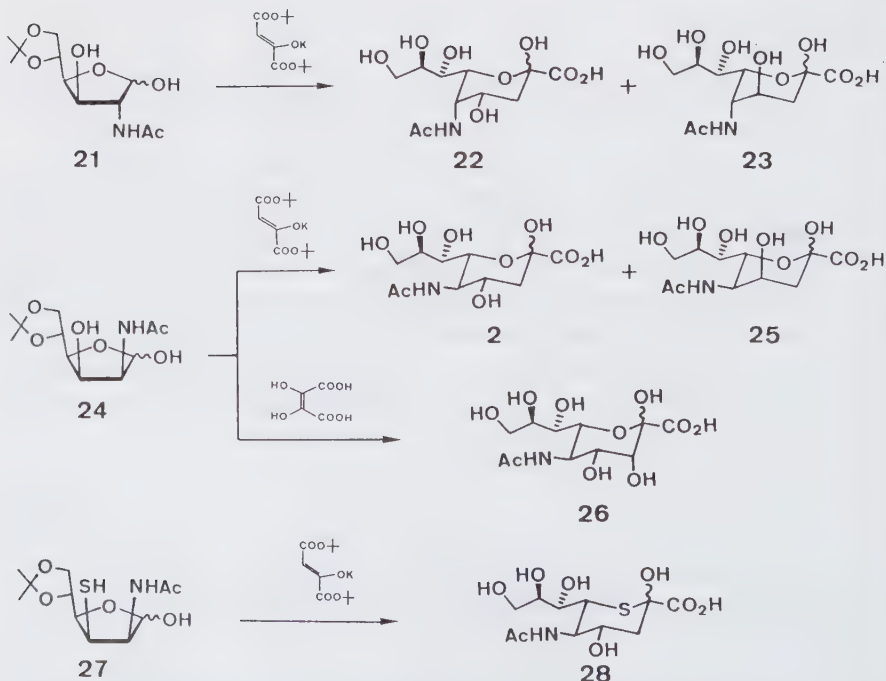
Kuhn and Baschang [72] synthesized Neu5Ac **2** in a yield of 30%. The key step of their synthesis was the reaction of an equilibrium mixture of 4,6-O-benzylidene-N-acetylglucosamine **18**⁵⁾ and 4,6-O-benzylidene-N-acetylmannosamine **19** (Scheme 4) with potassium di-tert.butyl oxaloacetate (**20**)⁶⁾ in dioxane-methanol 1:1. Neu5Ac was obtained after an acidic workup, followed by ion-exchange chromatography.

5) Available in good yields from GlcNAc and benzaldehyde/ZnCl₂ [73].

6) The potassium salt **20** was prepared in two steps from oxalic acid by its conversion into di-tert-butyl oxalate and subsequent reaction with t-BuOK and tert-butyl acetate (yield: 70%) [74][75].

The aldol reaction of **20** with various aldoses was also used for the syntheses of some Neu5Ac analogues. Gielen [76] synthesized an N-benzyloxy-carbonylneuraminic acid in a yield of 18% by reaction of N-benzyloxycarbonyl-D-mannosamine with potassium di-tert-butyl oxaloacetate (**20**) in dioxane-methanol. Similarly, N-benzoyl-, N-benzyloxycarbonyl- and N-ethoxy-carbonyl-neuraminic acids have been prepared by Wesemann and Zilliken [77] starting with the corresponding 4,6-O-benzylidene protected N-acyl-D-glucosamines and **20**. The sialic acids were obtained in yields of 20-24%. An 8-carbon analogue of Neu5Ac was obtained by base catalyzed reaction of di-tert-butyl oxaloacetate (**20**) with 2-acetamido-2-deoxy-D-lyxose in yields up to 29% [78].

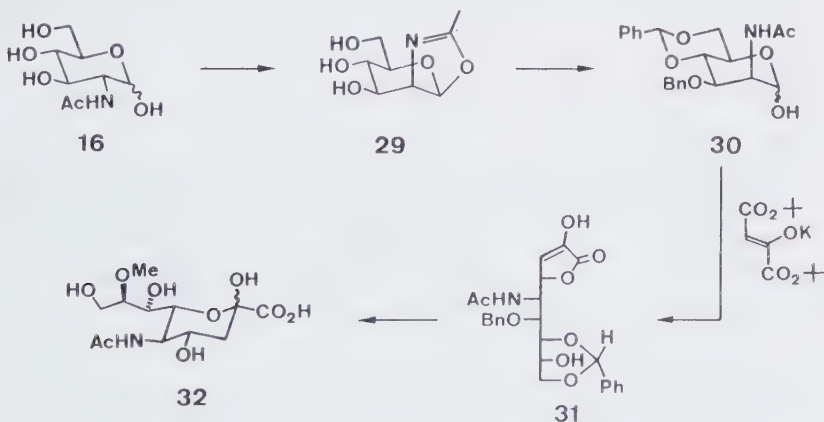
Scheme 5



Brossmer and coworkers [79][80] synthesized C(3), C(4), C(5) and C(6) analogues of Neu5Ac by aldol reaction of the furanoses 21, 24 and 27 with the oxaloacetate 20 in the presence of Ni^{2+} as catalyst (Scheme 5). These authors obtained a mixture of the D-glycero-D-gulo-isomer 22 in a yield of 40% and the D-glycero-D-ido isomer 23 in 60%. Similarly, 24 was converted into Neu5Ac 2 (32%) and 4-epi-Neu5Ac 25 (10%). Reaction of the furanose 22 with dihydroxy maleic acid produced under basic conditions the 3-hydroxy-Neu5Ac 26 in a yield of 20%. An epimeric mixture of N-acetyl-3-hydroxy-neuraminic acid had previously been synthesized by treatment of N-acetylmannosamine 5 with bromopyruvate followed by substitution of the bromo group [81]. Treatment of the thiofuranose 27 with the oxaloacetate 20 gave after deprotection and decarboxylation the 6-thio-sialic acid 28 (24%) [80].

In 1970, Khorlin and Privalova [82] described the synthesis of N-acetyl-8-O-methyl-neuraminic acid (32; Scheme 6). This sialic acid occurs naturally as a constituent of some glycolipids [83][84]. It was obtained from

Scheme 6

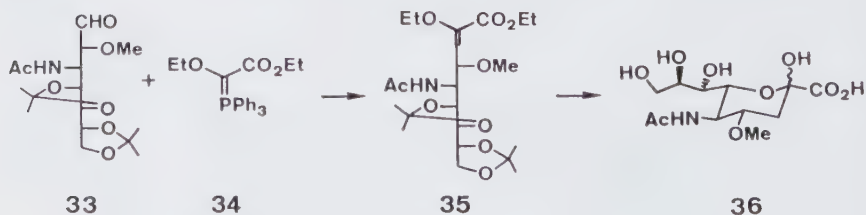


the γ -lactone 31 by methylation of the C(8) OH group, followed by deprotection. Nucleophilic addition of 20 to the N-acetylmannosamine derivative 30

was again the key step giving the γ -lactone **31**. The N-acetylmannosamine derivative **30** was prepared in a yield of 44% by benzylidenation and benzylation of the oxazoline **29**, obtainable from GlcNAc **16** by epimerisation to ManNAc **5** [69], treatment of ManNAc with AcCl/HCl and deacetylation (15%) [85]. The sialic acid **32** was obtained in an overall yield of 16% from **29** or ca. 1% from GlcNAc **16**. A synthesis of Neu5Ac allowing modifications at C(1)-C(3) has been reported by Perry and Benzing-Nguyen [86]. Their synthesis started with N-acetylmannosamine (**5**). Three chain elongations each time by a C-1-unit ($2 \times \text{CH}_3\text{NO}_2$ followed by a Nef-reaction, $1 \times \text{CN}^-$) gave the C-9-unit in 12 steps. This procedure was applied to the synthesis of $1\text{-}^{13}\text{C}$ -labeled N-acetylneuraminic acid, which was obtained in an overall yield of ca. 5% from ManNAc. The troublesome step of this synthesis was the first addition of nitromethane to ManNAc under alkaline conditions, which caused rapid epimerisation of ManNAc to GlcNAc.

N-acetyl-4-O-methyl-neuraminic acid **36** (Scheme 7) was synthesized by Schauer and Sinay [87-90] by an ethoxymercuration-demercuration reaction of the enol ether **35** followed by acidic deprotection. The enol ether **35** (30%) was obtained together with its 4-epi-derivative (22%) by a Wittig reaction between the protected *D*-glycero-*D*-galacto-heptose **33** and the ylide **34**.

Scheme 7

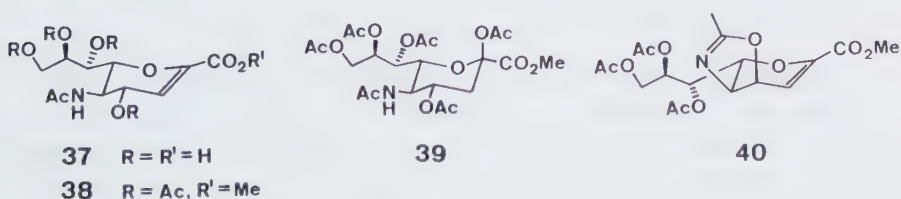


The heptose **33** was prepared from GlcNAc in ca. 6% by epimerisation of GlcNAc to ManNAc and a Nef-reaction giving preferentially a *D*-glycero-*D*-galacto configured heptose. This heptose was transformed into a dithioace-

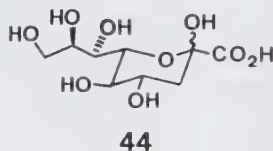
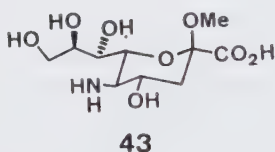
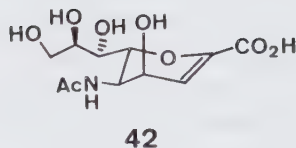
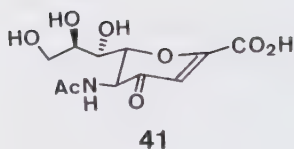
tal; isopropylidenation, methylation of the C(4) OH group and deprotection of the carbonyl function gave **33**. This synthesis yielded the sialic acid **36** in an overall yield of 14% from **33** or ca. 1% from GlcNAc.

1.5.3. Syntheses of Sialic Acids from N-Acetylneuraminic Acid

N-acetylneuraminic acid (**2**) can be isolated from edible bird's nest [91], colominic acid [92] or from the urine of sialuria patients [93]. Many sialic acids have been prepared from Neu5Ac **2**. Most of these Neu5Ac analogues were of interest for the study of sialidases. The enol ether **37** [94] has been known for quite some time. The corresponding peracetylated methyl ester **38** is a by-product of the Königs-Knorr glycosidation of methyl 4,7,8,9-tetra-O-acetyl- N-acetyl-2-chloro-2-deoxy-D-neuraminate [95]. Treatment of the peracetylated methyl N-acetylneuraminate **39** with trimethylsilyl triflate has been reported to give **38** in 90% [96]. The preparation of this compound has been examined by R. Julina [97]. In his hands the peracetylated Neu5Ac methyl ester **39** did not react under the conditions described by Claesson and Luthman [96]. Treatment of **39** with 2.0 eq. of TMSOTf in CH_3NO_2 , however, gave **38** in yields of 69% together with 15% of the oxazoline **40**.



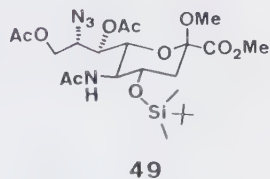
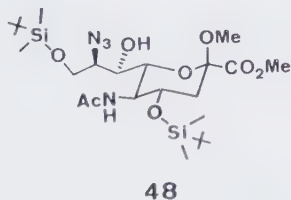
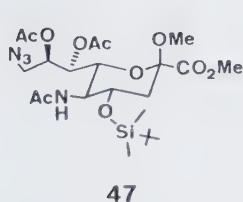
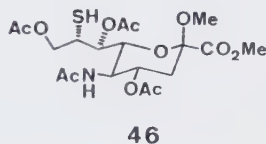
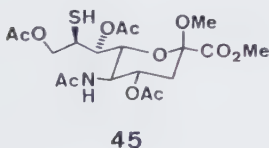
The 4-keto-sialic acid **41** has been prepared by manganese dioxide oxidation of a partially protected 2,3-dehydro-Neu5Ac derivative (15%) [98]. The C(4)-epimer **42** of 2,3-dehydro-Neu5Ac **37** was obtained either by treating methyl N-acetylneuraminate with $\text{Ac}_2\text{O}/\text{H}_2\text{SO}_4$ [99] or by treating methyl ester **38** with $\text{BF}_3\text{-Et}_2\text{O}$ followed by $\text{HOAc}/\text{H}_2\text{O}$ [100] and subsequent deprotection.



Some C(5)-analogues of Neu5Ac have also been prepared. The methyl β -D-glycoside of neuraminic acid **43** has been described by Gielen [101], who isolated it in 78% after hydrogenolysis of the corresponding Z-protected derivative. The glycoside **43** was also the intermediate in the syntheses of various N-acyl derivatives of Neu5Ac, e.g. N-[1- 13 C]-stearoylneuraminic acid [102], N-[1- 14 C]-glycolyl-, N-chloroacetyl- and N-fluoroacetyl-neuraminic acid [103], and N-[1- 14 C]-acetylneuraminic acid [104].

A deaminated sialic acid **44** has recently been isolated from a polysialoglycoprotein of rainbow trout eggs [105]. The structure of this nonulosonic acid has been confirmed by comparison with a chemically synthesized authentic sample [105], which was obtained by treating an N-glycolylneuraminic acid derivative with nitrous acid according to the procedure described by Mononen [106].

Sialic acids modified in the glycerol side chain are of interest due to their behaviour towards different sialidases and to N-acetylneuraminic acid synthetases. Zbiral and Brandstetter have synthesized a 7-epi-, 8-epi- and 7,8-bis-epi-N-acetylneuraminic acid [107]. Intermediates of these syntheses are 7,8-epoxy-Neu5Ac derivatives [108][109]. Other products of side chain variations are the 8-deoxy-8-mercapto-Neu5Ac derivatives **45** and **46** [110], as well as the 9-azido-9-deoxy-analogue **47**, and the 8-azido-8-deoxy-Neu5Ac derivatives **48** and **49** [111].



The 8-carbon- and 7-carbon-analogues of Neu5Ac were isolated after acid hydrolysis of *Collocalia mucoid*, which had been modified by periodate oxidation followed by borohydride reduction [78][112]. These analogues were also prepared by periodate oxidation and borohydride reduction of β -methoxy- N-acetyl-D-neuraminic acid methyl ester followed by acid hydrolysis. An other route to this compound is described in 1.4.2.

1.5.4. Enzymatic Syntheses of Sialic Acids

David et al. reported the enzymatic synthesis of Neu5Ac [113] and 9-O-acetyl-Neu5Ac [114] with acylneuraminate pyruvate-lyase (E.C.4.1.3.3) immobilized on agarose. Starting with a crude mixture of GlcNAc=ManNAc (16 and 5) and sodium pyruvate, Neu5Ac 2 was synthesized on a 2.8 mmol scale. Similarly, starting with 6-O-acetyl-N-acetyl-D-mannosamine, 9-O-acetyl-Neu5Ac was obtained on a 0.3 mmol scale. N-acetyl-9-azido-9-deoxy-neuraminic acid has been prepared by an enzymatic synthesis from N-acetyl-6-azido-6-deoxy-D-mannosamine and phosphoenolpyruvate with N-acetylneuraminate synthetase from *Neisseria meningitidis* 60E [115]. Analogous procedures have been applied earlier for the syntheses of N-[1-¹⁴C]-acetylneuraminic acid [116], N-acetyl-[1-¹⁴C]- and N-glycolyl-[1-¹⁴C]-neuraminic acid [117] and N-acetyl-[4-¹⁴C]-neuraminic acid [116]. (For a review see [118]).

2. Aim of the Project

The goal of the project was the development of a sialic acid synthesis, which would not only allow the preparation of Neu5Ac **2**, but also of analogues modified at any C-atom of the carbon skeleton. The total synthesis of Neu5Ac according to Danishefsky and DeNinno [64][65] (see Chap. 1.5.1) meets theoretically this condition, but the necessary procedures are not worked out.

Although the sialic acid synthesis basing on the aldol reaction of potassium di-tert-butyl oxaloacetate (**20**) and an aldose (as shown in Chap. 1.5.2) allows modifications at C(5) to C(9), only few such analogues have been prepared. Moreover, this procedure suffers from the lack of stability of 2-acetamido-hexoses possessing an axial substituent at C(2). Also, these compounds are only available in poor yields by procedures which usually involve an epimerisation at C(2) and several protection and deprotection steps.

Preparations of Neu5Ac analogues starting with N-acetylneuraminic acid require either the isolation of larger amounts of Neu5Ac from natural sources or a synthesis of it, followed in both cases by selective transformations (see Chap. 1.5.3). Although this approach is laborious, most of the analogues have been prepared this way.

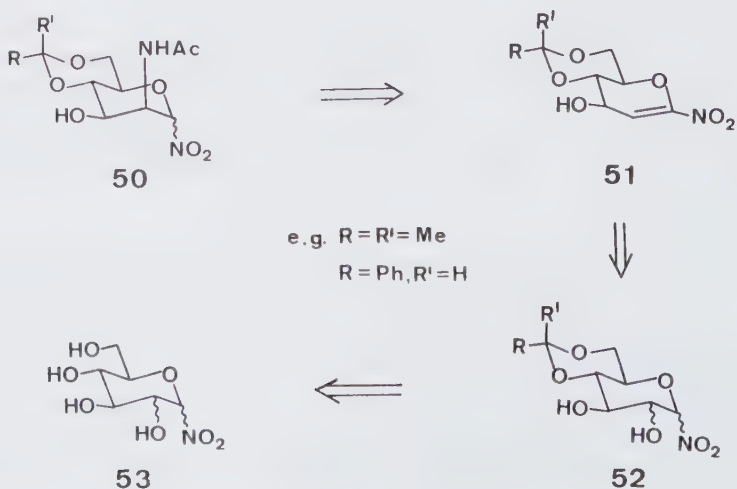
Enzymatic syntheses of sialic acids have mainly been applied for the preparation of isotopically labeled N-acetylneuraminic acid (see Chap. 1.5.4). The substrate specificities of the enzymes are not exactly known and structural modifications at C(1)-C(4) may be difficult using this procedure. On account of these restrictions, we wanted to reach the above goal by devising a chemical synthesis of sialic acids from cheap, commercially available carbohydrates.

Usually C-9-sugars are prepared by chain elongation of a pentose or of a hexose with a tetrose or with glyceraldehyde, respectively. In a sialic acid synthesis starting with a pentose and a C-4-unit either the C(4)-C(5)- or the C(5)-C(6)-bond must be formed. In both cases two new stereocentres have to be controlled in the C-C-bond forming step. In addition, stereoselective reactions are usually more easily controlled in hexoses than in pentoses. A chain elongation of a hexose by a C-3-unit follows the

biosynthesis of Neu5Ac, which obviously must be advantageous. Also, in forming the C(3)-C(4)-bond one only has to control the formation of one stereocentre. Therefore, we decided to chain-elongate a hexose by a C-3-unit. Such a hexose may possess either the normal carbonyl polarity at C(1) or the inverse one, as it is the case for 1-deoxy-1-nitro-aldoses. Sialic acid syntheses starting with hexoses of normal carbonyl polarity at C(1) are presented in Chap.1.5.2. Due to the above mentioned problems with such hexoses (especially epimerisation at C(2)), and the experience with 1-deoxy-1-nitro-sugars in our group, we decided to use these latter compounds. Both possibilities of a Neu5Ac synthesis starting with a 1-deoxy-1-nitro-hexose and a C-3-unit are depicted in the Schemes 9 and 10.

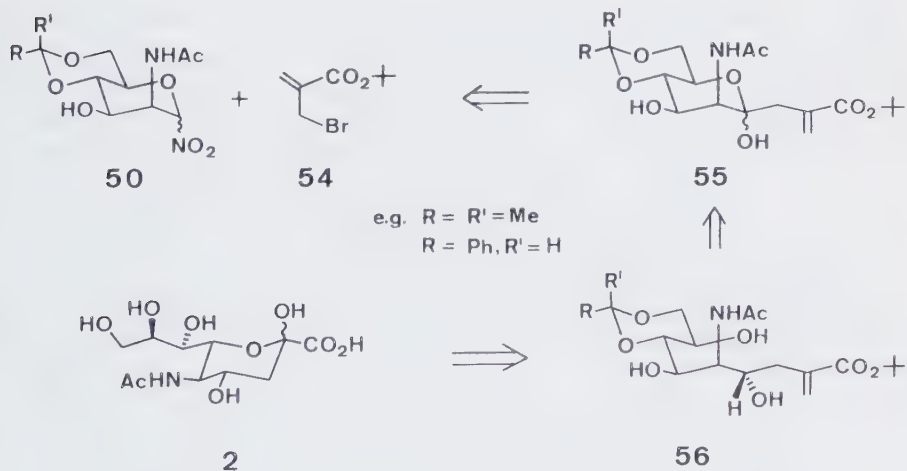
A prerequisite for a synthesis of N-acetylneuraminic acid according to Scheme 9 is a convenient approach to 1-deoxy-1-nitro-N-acetyl- D-mannosamine derivatives such as 50. Due to the poor availability of ManNAc 5, addition of NH_3 or amines to nitro olefins such as 51 (Scheme 8) appeared a more attractive route to a 1-deoxy-1-nitro-mannosamine derivative than a preparation from 5.

Scheme 8



This approach requires the preparation of nitro glycols 51, which might be new and generally useful intermediates, and the examination of some of their chemical properties. A first protected nitro glycol of this type has been prepared by Aebischer [119] and it seemed relatively easy to obtain such compounds. The nucleophilic addition to these nitro olefins, however, had not been examined with the exception of NaBH_4 reduction [119]. Stereoelectronic considerations (chair vs. boat transition state) favour an axial attack of the amine to the nitro olefin 51 leading to a D-manno-configured amino-nitro-sugar. To prepare the necessary nitro olefin 51, the C(2) hydroxy group of the precursor 52 has to be transformed into a leaving group [120]. It might be advantageous to use a partially protected nitro-hexose for this modification. According to [121], protected 1-deoxy-1-nitro sugars are conveniently available by ozonolysis of nitrones. Using an analogous procedure, unprotected nitro hexoses such as 53 might also be prepared. Since the stereocentre at C(2) is abolished in the β -elimination step either D-glucose or D-mannose are convenient starting materials.

Scheme 9



The reaction sequence as depicted in Scheme 10 requires reductive removal of the anomeric nitro group in 59. Methods for this displacement were known [128-134], but their diastereoselectivity was not clear. We, therefore, first investigated the stereoselectivity of the reductive denitration of easily available tertiary nitro ethers with Bu_3SnH in benzene (see Chap. 3). The further conversions of 60 into the 2,3-dehydro Neu5Ac derivative 61 and finally into Neu5Ac 2 should be possible using standard reactions.

The two possible reaction schemes 9 and 10 to prepare sialic acids allow for selective modifications at any one of the C-atoms of Neu5Ac.

C(1)-C(3) by applying modified Michael acceptors

C(4) and C(6) bear nitro groups, which can be transformed into other functional groups

C(5) is variable by addition of different nucleophiles to the nitro olefin

C(7)-C(9) can be modified by using different aldehydes.

The following chapters examine:

1. The reductive denitration of some tertiary nitro ethers (Chap. 3)
2. The preparation of 1-C-nitro glycals and the β -addition of ammonia to these nitro olefins (Chap. 4).
3. The synthesis of N-acetylneuraminic acid and N-acetyl-4-epi-neuraminic acid according to scheme 9 (Chap. 5).
4. The preparation of N-acetyl-4-deoxyneuraminic acid (Chap. 6).
5. The preparation of the 4-methylumbelliferyl α -D-glycoside of N-acetyl-4-deoxyneuraminic acid (Chap. 7).
6. The syntheses three 6-amino-6-deoxy-sialic acids (Chap. 8).

3. Stereoelectronic Control in the Reductive Denitration of Tertiary Nitro Ethers. A Synthesis of 'C-Glycosides'

3.1. Introduction

Tertiary nitroalkanes are reductively denitrated by treatment with the sodium salt of methanethiol [128], with ethylene glycol and KOH [129], with N-benzyl-1,4-dihydronicotinamide [130] or with tributyltin hydride (Bu_3SnH) [133][134]. As far as it is known, these reactions follow a radical-chain mechanism and proceed via planar radical intermediates. As expected, the few stereochemically relevant reductions so far studied occur without stereospecificity [128] and with a variable diastereoselectivity [132] which is difficult to predict⁸⁾.

In the case of conformationally biased α -nitro ethers, however, the analogous reductive denitrations are expected to occur with a high degree of diastereoselectivity.

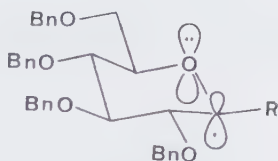
It is known that the intermediates, viz. alkoxyalkyl radicals, are pyramidal [137-138] and that the non-bonded C-orbital prefers an orientation which allows conjugative delocalization⁹⁾ with the π -type orbital on the O-atom [140], i.e. a (pseudo)axial orientation in 1-oxacycloalk-2-yl radicals [141][142]. This is substantiated by a stereoelectronic preference for the radical abstraction of (pseudo)axial α - and β -H-atoms in conformationally biased cyclic ethers [139][141][143-144]¹⁰⁾. The same stereoelectronic effect should also control the reverse reactions and this has been shown for the H-transfer to 1,3-dioxanyl radicals [144]

⁸⁾ The steric course of the similar reduction of thionoesters [132-136] and tertiary benzoates [137] by Bu_3SnH has been explained by steric and polar factors.

⁹⁾ See [139] and ref. cited therein.

¹⁰⁾ A similar stereoelectronic control in the cleavage of a C,O-bond is described in [145].

We wished to check, if the radical denitration with Bu_3SnH of carbohydrate nitro ethers such as **64** and **65** (Scheme 11) will indeed diastereoselectively lead (via a common intermediate, e.g. **62**) to the corresponding anhydroalditol such as **66** with two equatorial side chains¹¹⁾. Further insight into the stereoselectivity of this reaction was expected from the reduction of conformationally biased nitrocyclohexanes (Scheme 12) and from conformationally more flexible furanose derivatives (Scheme 13 and 14). Since such tertiary carbohydrate nitro ether are easily available [122] from 1-deoxy-1-nitroaldoses [121][147] via Michael additions or via the Henry reaction [126], the reductive denitration would open a way to 'C-glycosides' (=anhydroalditols) which recently have attracted much interest (see e.g. [148-149] and ref. cited therein).



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3.2. Results

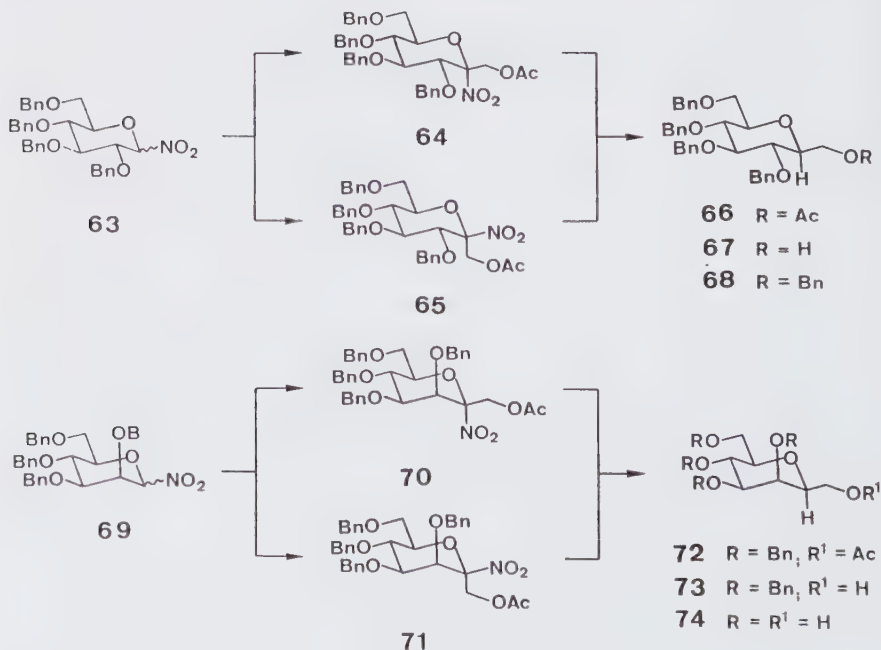
Base-catalysed reaction of the D-gluco-deoxynitroaldose **63** [121][147] with excess paraformaldehyde followed by acetylation gave the nitro ethers **64** and **65** in a yield of 56% and with a ratio of 85:15 ($^1\text{H-NMR}$; Scheme 11)¹²⁾. The isomers were separated by prep. HPLC. Their IR spectra sho-

11) A detailed study by Giese and coworkers has shown that this approach is too simple and that the conformation of the radicals depends on their configuration. For a leading ref. see [146].

12) Partial solvolytic displacement of the NO_2 -group occurred during the reaction, see [122].

wed NO_2 -bands at 1552 cm^{-1} (major compound) and 1566 cm^{-1} (minor compound); the assignment of the anomeric configuration was based on the observation that the IR absorption of an axial NO_2 -group of secondary nitro ethers is shifted to lower wave numbers [147]. Separate treatment of **64** and **65** with Bu_3SnH in the presence of α, α' -azoisobutyronitrile (reflux in benzene) gave the 2,6-anhydroalditol **66** in yields of 95 and 87%, respectively, and traces of minor compounds that were not isolated. The configuration of **66** was proven by its transformation into the alcohol **67**¹³) and further into the meso-pentabenzyl ether **68**. The meso nature of **68** was evident from its ^1H - and ^{13}C -NMR spectra.

Scheme 11

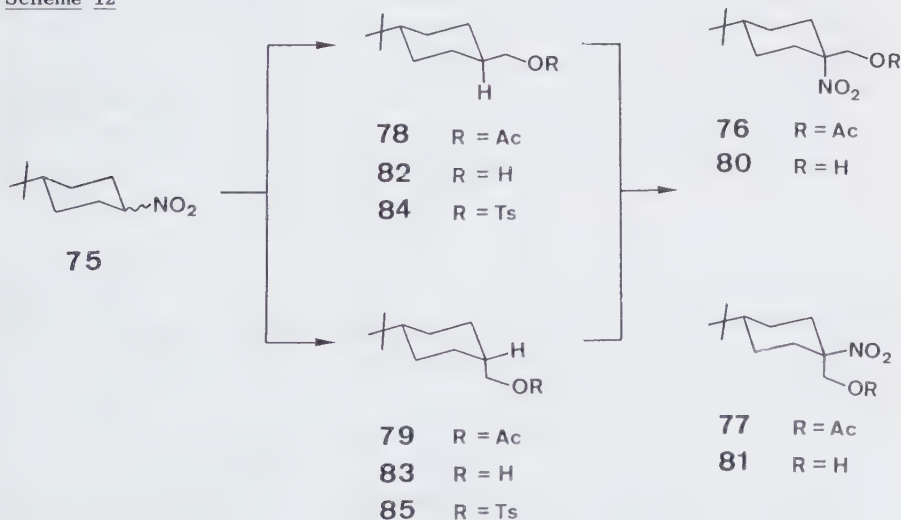


¹³) The ^1H -NMR spectra and the specific rotation were different from those of the anomeric alcohol obtained by Sinaÿ et al. [149]. We thank Prof. Dr. P. Sinaÿ for the relevant spectra.

The similar reaction sequence starting with the D-manno-deoxynitroaldose 69 [121][147], gave the tertiary nitro ethers 70 (65%) and 71 (12%). Again, separate treatment of 70 and 71 with Bu_3SnH , as above, gave the protected 2,6-anhydro-D-glycero-D-galacto-heptitol 72 as the only observed isomer. Deprotection of 72 (deacetylation to 73 followed by hydrogenolysis) gave the known [150], free anhydroheptitol 74.

To evaluate the influence of the ring-O-atom on the diastereoselectivity of the reduction, we treated the tertiary nitro compounds 76, 77, 80, and 81 with Bu_3SnH under the same conditions as above (Scheme 12). The crystalline nitro alcohols 80 and 81 were obtained (>95%, 7:3) from 1-(tert-butyl)-4-nitrocyclohexane (75) [151][152] and acetylated to give 76 and 77. Their configuration was inferred from the ^1H -NMR spectra. The signals of the equatorial H-C(2 and 6) were very similar to those found for the corresponding H-atoms of 75 and were interpreted according to Huitric and Trager [151].

Scheme 12



Separate reduction of the acetates 76 and 77 gave a mixture of the denitrated acetates 78 and 79 in a high yield and with a ratio of 83:17, as

determined by ^1H -NMR spectroscopy¹⁴⁾ and GC. Similarly, the separate reduction of the alcohols 80 and 81 gave a 4:1 mixture (^1H -NMR) of 82 and 83 [154] in over 95%. The acetates 78 and 79 and the alcohols 82 and 83 could not be separated, but pure samples of the trans- and cis-tosylates 84 and 85 [155] were obtained by HPLC.

The behaviour of five-membered, tertiary nitro ethers was checked for trioxabicyclo[3.3.0]octane derivatives of the D-manno¹⁵⁾ - and D-ribo-series (Scheme 13 and 14). Compound 86 [122] upon reduction with Bu_3SnH , gave the 4,7-anhydroaldonitrile 87 as the main product (58%)¹⁶⁾. The configuration at C(4) of 87 was deduced from its ^1H -NMR spectrum ($J_{4,5}=3.8$ Hz [156]). Similar reduction of the dodecosulose 88 [122] gave (89%) the anhydro compound 89 as a single, crystalline isomer. Here again, the value of 3.7 Hz for $J_{7,8}$ is consistent with a 7,8-cis-configuration (signal assignment by ^1H -shift-correlated 2-D NMR spectroscopy). Separate reduction of the psicofuranose derivatives 91 and 92 (from 90, see [122]), however, gave a 47:53 mixture¹⁷⁾ of the allo- and alto-anhydroalditols 93 and 94 in a combined yield of 90 and 87%, respectively (Scheme 14). Their configuration was determined by transformation of 93 into the meso-diacetate 97 and of 94 into the known [157] diacetate 98. This was achieved by detritylation (FeCl_3 [158], 78%) of the mixture of 93/94, separation of the alcohols 95 and 96, and then, on the one hand, back-tritylation to pure 93 and 94 and, on the other hand, acetylation to 97 and 98.

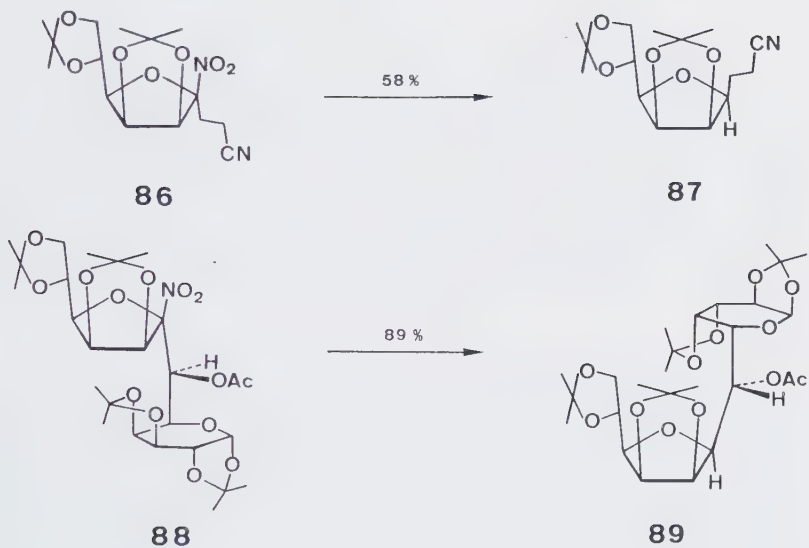
14) Based on the integrals for the acetoxymethyl group. Both diastereomers had been prepared and characterized [153].

15) Configurational prefix refers to the furanose ring only.

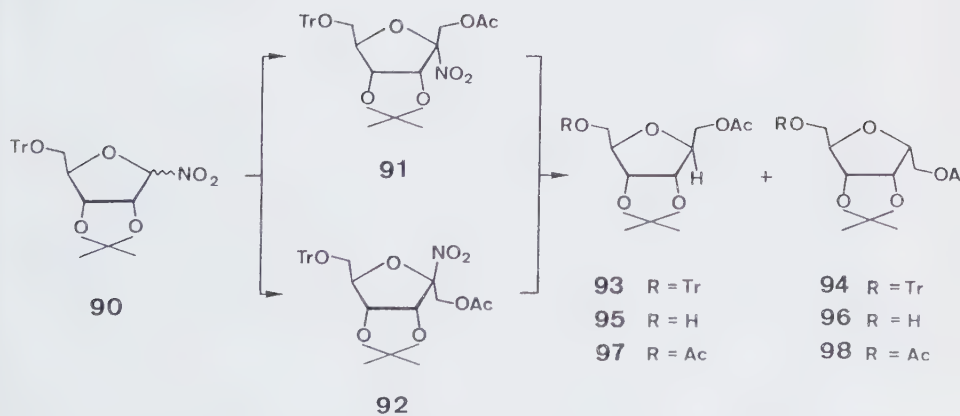
16) The IR spectra of three by-products were not consistent with the structure of diastereoisomers.

17) The ratio was determined by HPLC. The same ratio of products was obtained from a reduction of 91 with Bu_2SnH_2 .

Scheme 13



Scheme 14



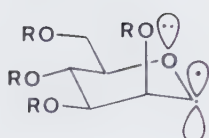
3.3. Discussion¹⁸⁾

The reductive denitration of the conformationally biased, tertiary nitro ethers of the pyranose series leads to 'C-glycosides' in good yield and - as expected - with a diastereoselective formation of an axial C,H-bond. In the furanose series, the explanation of the diastereoselectivity requires an evaluation of both steric and stereoelectronic factors. Since the tertiary nitro ethers are obtained from carbohydrate derivatives with inverse polarity at the anomeric center [122], this access to 'C-glycosides' complements earlier methods.

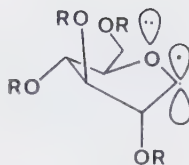
The assumption of a common intermediate - an alkoxyalkyl radical such as 62 - corresponds with the formation of the same product(s) from either anomeric starting material. The high tendency for the axial C,H-bond formation in cyclohexanes makes it difficult to evaluate the extent of the stereoelectronic effect of the ring-O-atom. The stabilizing conjugative interaction in the tetrahydropyran-2-yl radicals seems to be rather weak (from the degree of pyramidalization at C(1) as deduced from the hyperfine coupling constants, see [139]) and a similar reactivity of the cyclohexyl- and tetrahydropyran-2-yl radicals has been proposed [139]. The influence of the axial configuration of the vicinal alkoxy substituent in 70 and 71 is not required for the diastereoselectivity.

As have been shown by Giese et al. [146], the conformation of glycosyl radicals depends on their configuration. Whilst the mannosyl radical 99 remains in the chair conformation, the glucosyl radicals do not adopt the conformation of their precursors, but change to a boat conformation such as 100. In both cases the β -C-O-bond is axial. This leads to a favorable anti attack with respect to the β -C-O-bond at the radical centre. Hence, products with an axial C-H-bond have to be expected in the gluco- and manno-series.

¹⁸⁾ We thank Prof. Dr. H. Fischer and Dr. H. Paul for helpful discussions.



99



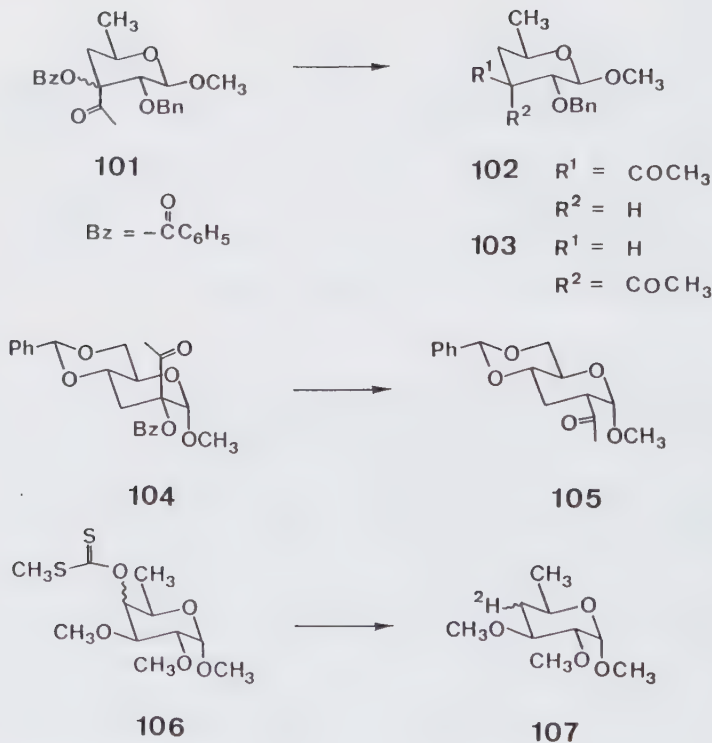
100

R = protectiv group

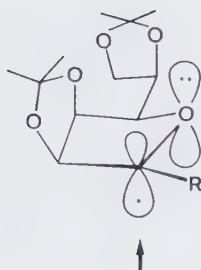
2-Alkoxyalkyl radicals prefer a coplanar arrangement of the non-bonded, singly occupied orbital and the C,O-bond [159]. This effect can influence the steric course of radical reductions as inferred from the following examples¹⁹⁾. Reduction of either epimer of 101 (Bu_3SnH) gave a 4:1 mixture of 102 and 103 [137] (Scheme 15; the coplanar arrangement mentioned cannot be attained without ring distortion; the steric behaviour is very similar to the cyclohexyl case). Reduction of 104 gave 105 exclusively [137] (additional influence of the axial C(1),O-bond). Reduction of either epimer of 106 resulted in a 1:1 mixture of the epimeric, monodeuteriated reduction products 107 [136] (coplanar arrangement of the non-bonded C-orbital and the C(5),O-bond and preference for an attack anti to the C,O-bond against the tendency for axial C,O-bond formation).

¹⁹⁾ For the discussion of the stabilizing effect of β -alkoxy groups on C-radicals see [160].

Scheme 15

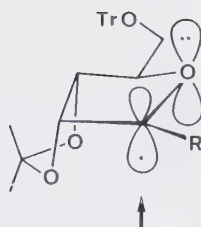


The relatively high proportion of endo-attack by Bu_3SnH on the intermediate D-ribo-radical is surprising, particularly when compared to the results in the D-manno-series. This can be explained as the result of a steric effect, favouring exo-attack and a stereoelectronic effect, favouring endo-attack in the D-ribo and exo-attack in the D-manno-series. .



D - manno

108



D - ribo

109

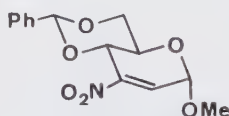
The stereoelectronic effect depends on the conformation of the furanose-ring in the intermediate radical²⁰). This conformation is dominated by the preferred pseudoequatorial orientation of the endo-dioxolanyl side chain in the D-manno-radical 108 and the exo-trityloxymethyl side chain in the D-ribo-radicals 109 (cf. [156]). The different conformation of the furanose-ring in 108 and 109 entails pyramidalization of the non-bonded C-atom in the endo-direction in the D-ribo-series and in the exo-direction in the D-manno case. This means that the stereoelectronic effect is at least as strong as the steric bias for exo-attack on a trioxabicyclo[3.3.0]octane system assuming the extreme case of conformational homogeneity around the radical center.

²⁰) Assuming both kinetic control and a low activation energy for H-transfer from Bu₃SnH (2.95 to 3.97 kcal/mol for alkyl radicals [161]).

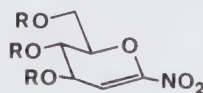
4. 1-C-Nitroglycals: Synthesis and Reaction with Nitrogen Nucleophiles

4.1. Introduction

α,β -unsaturated 3-C-nitro olefins, e.g. **110**, have been used for the synthesis of deoxy-, aminodeoxy-, and branched-chain sugars [162]. As illustrated in Chap. 2, a 1-C-nitroglycal such as **111** might be a starting compound for the synthesis of 1-deoxy-1-nitro-mannosamine derivatives.



110



111

R = protective group

In spite of the donor effect of the ring oxygen of a 1-C-nitroglycal, we expected a stereoselective (axial) addition of nucleophiles to the C,C double bond. An advantage of this way to ManNAc derivatives is the possibility of modifying the glycal before addition of NH_3 . The C(3)-OH group of **111** is an allylic one and may easily be converted into other functional groups. This OH group corresponds to the C(6)-OH group in Neu5Ac (following Scheme 9). Thus, a modification at C(3) of **111** leads to a C(6) analogue of Neu5Ac. As will be demonstrated in Chap. 8, this strategy was used to synthesize 6-amino-6-deoxy-sialic acids.

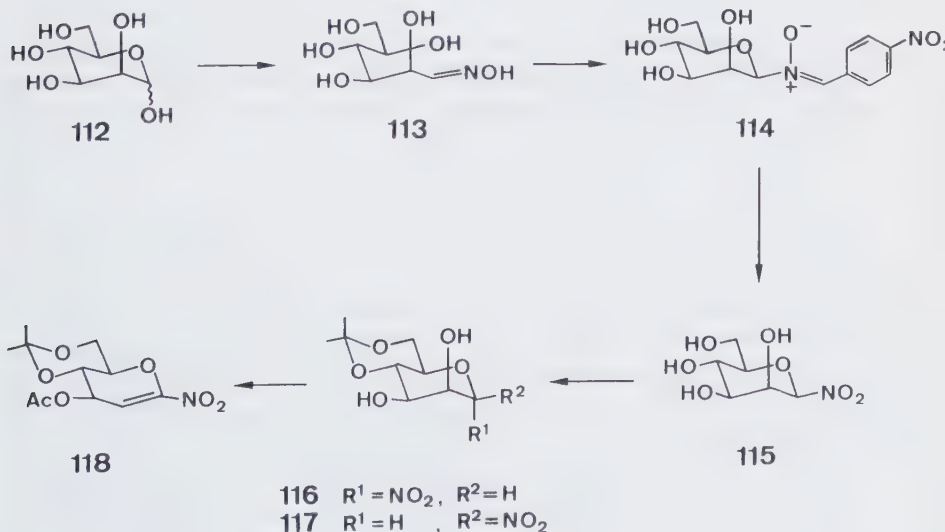
A prerequisite for the synthesis of **111** from a hexose is the transformation of the C(2)-OH group into a leaving group. The acetoxy group fulfills this condition [120][163]. The remaining OH groups (at C(3), C(4) and C(6)) should be protected in order to allow selective reactions. Unprotected 1-deoxy-1-nitro-hexoses seem to be suitable intermediates for obtaining such a compound, since 4,6-O-protective groups can easily be introduced and the reactivity of the C(1)-OH group has not to be taken into account. We planned to synthesize the unprotected 1-deoxy-1-nitro-D-mannose **115** according to the procedure developed by Aebischer and Vasella [121][147] for the preparation of protected nitrosugars.

The syntheses of differentially protected 1-C-nitroglycals from 1-deoxy-1-nitro-D-mannose and 1-deoxy-1-nitro-D-glucose, and the β -addition of ammonia to them is described. The preparation of unprotected 1-deoxy-1-nitro-pyranoses and their subsequent conversion into 1-C-nitroglycals was simultaneously investigated more thoroughly by D. Beer and will be presented in his dissertation [164].

4.2. Results

By analogy to [121], treatment of D-mannose **112** with H_2NOH gave the crystalline D-mannoseoxime **113** [165] (quant. yield, Scheme 16). The oxime **113** was converted into a unique crystalline nitrone **114** (91%), which upon ozonolysis was transformed into crystalline 1-deoxy-1-nitro-D-mannose **115**.

Scheme 16

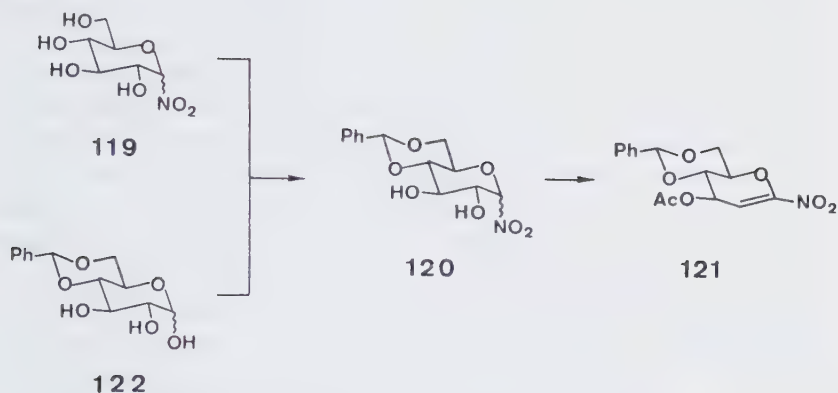


The formation of the oxime 113 and of the nitrone 114 proceeded without difficulties. The isolation of the nitro mannose 115 caused problems due to the instability of 115 in H₂O and its low tendency to crystallize. According to its ¹³C-NMR spectra, 113 was in the open-chain oxime form [164][166]. The ring size of the nitrone 114 and of the 1-deoxy-1-nitro-hexose 115 was assigned from δ -C(1) in their ¹³C-NMR-spectra. The values of 95.3 ppm (114) and 102.8 ppm (115) are typical for 1-deoxy-1-nitro-pyranoses [164][166]; 1-deoxy-1-nitro-furanoses have δ -C(1)-values of ca. 108 ppm [166]. The β -D-configuration of 114 and 115 was deduced from the J_{1,2}-values (1.0 Hz for 114, 1.4 Hz for 115). These values were compared with those of earlier synthesized 1-deoxy-1-nitro-D-mannopyranose derivatives [147][167], showing that β -D-configured mannopyranoses have J_{1,2} $\leq$ 1.5 Hz and α -D-configured ones have J_{1,2} $>$ 2.1 Hz [164][166]. The Z-configuration of the nitrone 114 was determined by Nuclear Overhauser Enhancement (19%) between the H-C(1) and the benzylic H-atom [164][166].

Acid catalyzed conversion of 115 with 2-methoxy-propene in DMF produced the anomeric 4,6-O-isopropylidene derivatives 116/117 [168][169]. The best yield of 116/117 was obtained when the nitrone 114 was directly transformed into 116/117 without isolation of the 1-deoxy-1-nitro-D-mannose (115; 66% from 114). A singlet at 98.8 ppm and two quartets at 28.1 ppm and 18.2 ppm in the ¹³C-NMR-spectrum of 116/117 confirm the presence of a 1,3-dioxane ring [170].

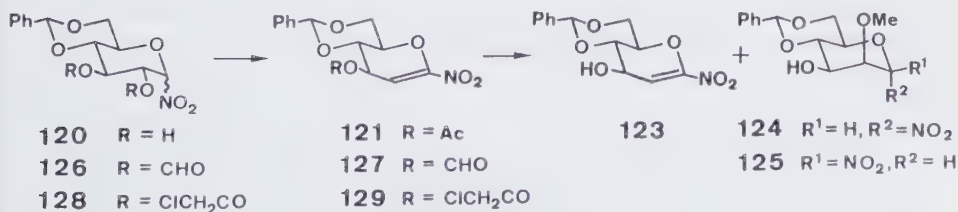
Acetylation of 116/117 (Ac₂O, pyridine, 0°) gave unstable intermediates (TLC), which were neither isolated nor characterized. Their partially spontaneous reaction to the 1-C-nitroglycol 118 was completed by mild base treatment of the reaction mixture. In a similar way, Christen [171] has synthesized the 4,6-O-benzylidene-1-C-nitroglycol 121 from the unprotected 1-deoxy-1-nitro-glucose 119 [166] in a yield of 41%, while Harder and Ganguly (cf. [166]) in their synthesis of 121 started with 122 [172] and introduced the nitro function in the usual way [121] (55%, Scheme 17). The partly protected nitroglucose 120 is an intermediate, which is common to both syntheses. The overall yield of the isopropylidene derivative 118 from D-mannose was 45%, while the benzylidene glycol 121 was best obtained in 33% from D-glucose [166].

Scheme 17



The desired conversion of the C(3)-OH group into other functional groups (see above), requires a selective deprotection of the glycals 118 and 121. Deacetylation of 121 with 0.5 mol equiv. NaOMe in MeOH at -30° (1 h) gave the nitro olefin 123 in 84% and an anomeric mixture of the crystalline 2-methoxy-D-manno-pyranoses 124/125 in 4% yield besides traces of the starting compound 121 (Scheme 18). The yields of this deacetylation depend strongly on the reaction conditions. Even slightly higher temperatures or increased reaction times led to lower yields of the nitro olefin 123 and to an increased yield of the methanol addition product 124/125 [171][173].

Scheme 18



We, therefore, looked for other routes to the nitroglycal 123 (Scheme 18). Formylation of 4,6-O-benzylidene-1-deoxy-1-nitro-D-glucose (120) with $\text{Ac}_2\text{O}/\text{HCOOH}$ 2:5 [174][175] gave 126 as an intermediate, which upon β -elimination of formic acid promoted by Amberlite IRA-93 (CHCl_3 , 60°), gave the crystalline nitro olefin 127 (83% from 120). Similarly, chloroacetylation of the protected 1-nitro-glucose 120 with chloroacetic anhydride and pyridine in ether gave the chloroacetylated nitrosugar 128 in almost quantitative yields²¹). For the β -elimination of chloroacetic acid, 128 was treated with Amberlite IRA-93 in benzene at 60° . This gave the crystalline nitro olefin 129 in 54% yield²²). Deformylation of 127 (NaOMe/MeOH , THF, 0°) yielded 123 (82%). Deacetylation of 129 with sodium m-nitrophenolate (0.10-0.15 moleq.) in MeOH (1 h) at 0° gave the crystalline nitro olefin 123 in a yield of 93%. Comparison of the three syntheses to produce 123 shows that the best yields were obtained by formylation/deformylation (68% from 120), while acetylation/deacetylation and chloroacetylation/deacetylation gave 57% and 50%, respectively.

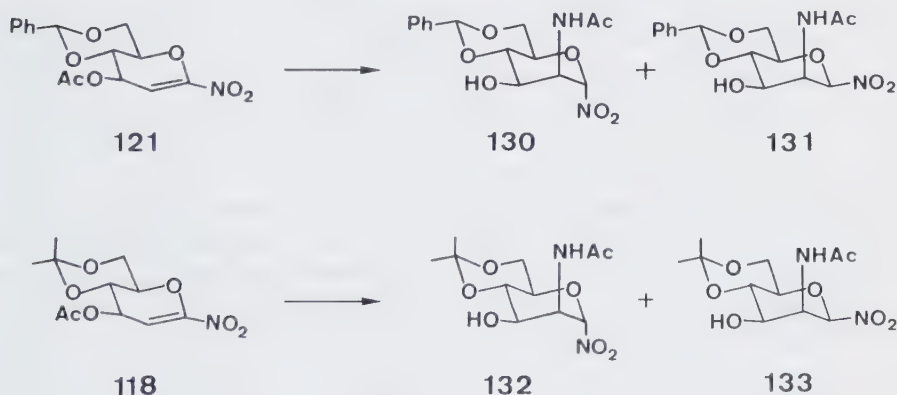
Due to the α -alkoxy substituent, the UV-maxima of the conjugated nitro olefins 118 and 123 showed a bathochromic shift of ca. 35 nm to 285 nm (ϵ 3500). The IR-spectra of 118 and 123 show absorptions at ca. 3100 cm^{-1} ($\text{C}=\text{CH}$), 1670 cm^{-1} ($\text{C}=\text{C}$) and 1550 cm^{-1} (NO_2). In the ^1H -NMR-spectra of 118 and 123 the doublet of H-C(2) appears at about 6.3 ppm ($J_{2,3} = 2.5$ and 2.8 Hz, respectively). In the ^{13}C -NMR-spectra, 118 and 123 are characterized by a singlet for C(1) at 153.35 ppm (118) and 151.5 ppm (123), respectively, and a doublet for C(2) at 100.55 ppm (118) and ca. 106 ppm (123).

21) Initial experiments to synthesize 127 and 129 were performed by Werner Altherr (undergraduate research project).

22) Other conditions for β -elimination like IRA-93/ CHCl_3 or K_2CO_3 /benzene or NEt_3 /benzene did not give better yields. Conditions using nonnucleophilic bases were not tested.

The planned synthesis of Neu5Ac, as indicated in Chap. 2, and described in Chap. 5, requires a convenient preparation of a N-acetyl-1-deoxy-1-nitro-mannosamine derivative. To ward this goal, we added NH_3 to the glycals 118 and 121. Best results were found by treating the nitro olefins with 25% aq. NH_3 in THF giving the anomeric ManNAc derivatives 130/131 and 132/133 respectively, in yields of 93% and 80% (Scheme 19). The ratio of the α : β anomers was in both cases 85:15 (^1H -NMR).

Scheme 19

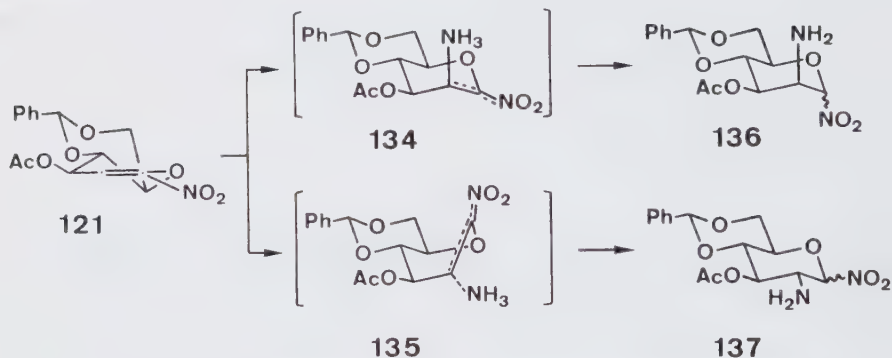


IR-absorptions at 1670 cm^{-1} and 1565 cm^{-1} point to the presence of an acetamido and nitro group. The former is also found in their ^1H -NMR spectra. The manno-configuration of 130/131 and 132/133 is assigned from $J_{1,2}$ and $J_{2,3}$ in their ^1H -NMR-spectra. The α -D-anomers 130 and 132 have $J_{1,2}$ -values of 0-1 Hz, the β -D-anomers 131 and 133 such of 0-2.2 Hz. In all cases $J_{2,3}$ was ca. 5.0 Hz.

The ManNAc derivatives 130/131 and 132/133 are the products of an axial attack of NH_3 to the C,C double bond of the nitro olefins 118 and 121. This attack was followed by an O $\rightarrow$ N acetyl migration [176][177]. The axial attack of NH_3 is not surprising, since attack from above the molecular plane leads

to a chairlike transition state **134**, whereas attack from below leads to a boatlike transition state **135** (Scheme 20).

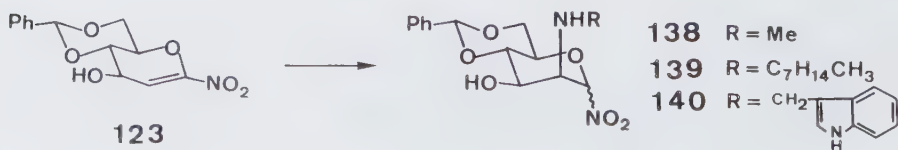
Scheme 20



The chair-conformationed **134**, leading to the **D-mannosamine derivative 136**, is energetically more favorable than the boat-conformationed **135**, which leads to the glucosamine derivative **137**. Thus, 1,4-addition of NH_3 or amines to the nitroglycals **118**, **121** or **123** should predominantly give mannosamine derivatives.

Christen [171] has found that methylamine, octadecylamine and tryptamine add also to the nitroglycal **123**. The corresponding crystalline mannosamine derivatives **138**, **139** and **140** were obtained in yields of about 75%. Addition of these amines to **11**, however, gave unsatisfactory results. These syntheses of **D-mannosamine derivatives** illustrate a new way to 1-deoxy-1-nitro-N-acetyl-D-mannosamines.

Scheme 21



5. Synthesis of N-Acetylneuraminic Acid and N-Acetyl-4-epineuraminic Acid

5.1. Introduction.

As presented in Chap. 2, we wished to work out a synthesis of N-acetylneuraminic acid (**2**), which should allow the preparation of its analogues modified at any C-atom of the carbon skeleton, aiming first at a modification at C(4). Analogues of Neu5Ac modified at C(4) are of interest in the context of the mechanism of neuraminidases. The N-acetyl-2-deoxy-4-epi-neuraminic acid [178], the N-acetyl-2,3-didehydro-4-epi-neuraminic acid, its methyl ester [99] [179] and N-acetyl-2,3-didehydro-4-oxoneuraminic acid [99] were all competitive inhibitors of *Arthrobacter sialophilus* neuraminidase [179] and of the influenza viral neuraminidase [179]. Bacterial and mammalian sialidases were completely inactive to the naturally occurring N,4-O-diacetylneuraminic acid [180][181], which was also hardly cleaved by acylneuraminate pyruvate-lyase [182]. Furthermore, the synthetic N-acetyl-4-O-methylneuraminic acid was resistant to acylneuraminate pyruvate-lyase and did not inhibit the aldol cleavage of **2** by this enzyme [90]. (N-acetyl-4-O-methylneuraminic acid)fetuin (prepared from N-acetyl-4-O-methylneuraminic acid and asialofetuin with the help of sialyltransferase [183]) releases N-acetyl-4-O-methylneuraminic acid upon treatment with fowl-plague neuraminidase [90], but is strongly resistant to *Vibrio cholerae* neuraminidase [90].

A synthesis of N-acetylneuraminic acid (**2**) and of its 4-epimer (**159**) (Scheme 22) is described. The reaction sequence bases upon the Michael addition of a partially protected 2-acetamido-1,2-dideoxy-1-nitro-D-mannose to a 2-(bromomethyl)acrylate, followed by a β -elimination as the key transformation.

5.2. Results.

Two derivatives of 1,2-dideoxy-1-nitro-D-mannopyranose, viz. the 4,6-O-benzylidene acetal 130/131 and the 4,6-O-isopropylidene acetal 132/133²³) ([166], Chap. 4) were subjected to base catalyzed addition to tert-butyl 2-(bromomethyl)prop-2-enoate²⁴) (54) [184], [185]. Michael addition of 130/131 to the tert-butyl acrylate 54 (DBU, THF, 0°) followed by carefully controlled hydrolytic removal of the NO₂ group [122] gave a 65:35 mixture of the benzylidene-protected 4-nonulosonate tautomers 143 and 144 (64%). Similarly, 132/133 reacted with 54 to give, after hydrolysis, a 65:35 mixture of the isopropylidene-protected 4-nonulosonate tautomers 145 and 146 (83%; Scheme 22)²⁵).

In the ¹³C-NMR spectra ((D₆)DMSO), the major (hydroxy ketone) isomers 143 and 145 show C(4)-carbonyl signals at 206.07 and 206.17 ppm, respectively. The C(4) signals of the hemiacetal isomers 144 and 146 appear at 99.44 and 99.39 ppm, respectively. These are typical values for α-D-anomers [186]. Signals of the β-D-anomers were not observed. The

²³) The overall yield of 132/133 from D-Mannose (35%) ([166], Chap. 4) was not affected by upscaling; most intermediates crystallize easily. The overall yield of 130/131 from D-Glucose was 28% for batches up to 10 g ([166], Chap. 4); batches of 50 to 80 g gave a slightly lower yield (21%), mainly due to the formation of by-products during ozonolysis and the chromatographic purification of 1-Deoxy-1-nitro-D-glucose.

²⁴) We thank Dr. H. Braunschweiger, Sandoz AG, Basel for a generous gift of this compound.

²⁵) Both the reactions of 130/131 and 132/133 with 54 gave a main product and a by-product. The IR spectra of the crude products from 130/131



ratios of 143/144 and 145/146 were determined by integration of the NH signals in the ^1H -NMR spectra ((D_6) DMSO) and confirmed by the integrals in the ^{13}C -NMR spectra. The same ratio of 143/144 was observed for a (D_8) dioxane- D_2O (4:1) solution even after addition of 2 equiv. of AcOD. A 65:35 ratio of 145/146 was also observed for a CDCl_3 solution.

Reduction of a mixture 143/144 with NaBH_4 in dioxane- H_2O (4:1) in the presence of 2 mol-equiv. of AcOH ²⁶⁾ gave a 94:6 mixture of the epimeric triols 147 and 148, which were separated by medium-pressure liquid chromatography (MPLC) [190] (92% combined yield). Similarly, reduction of 145/146 gave a 92:8 mixture of 149 and 150 (83%). Their tri-O-acetates 151 and 152 were separated by MPLC and characterised. The configurations of the epimeric triols 147/148 and 149/150 were determined by transformation of 147 and 149 into N-acetylneuraminic acid (2) and by transformation of 148 and 150 into N-acetyl-4-epineuraminic acid (159) as detailed below.

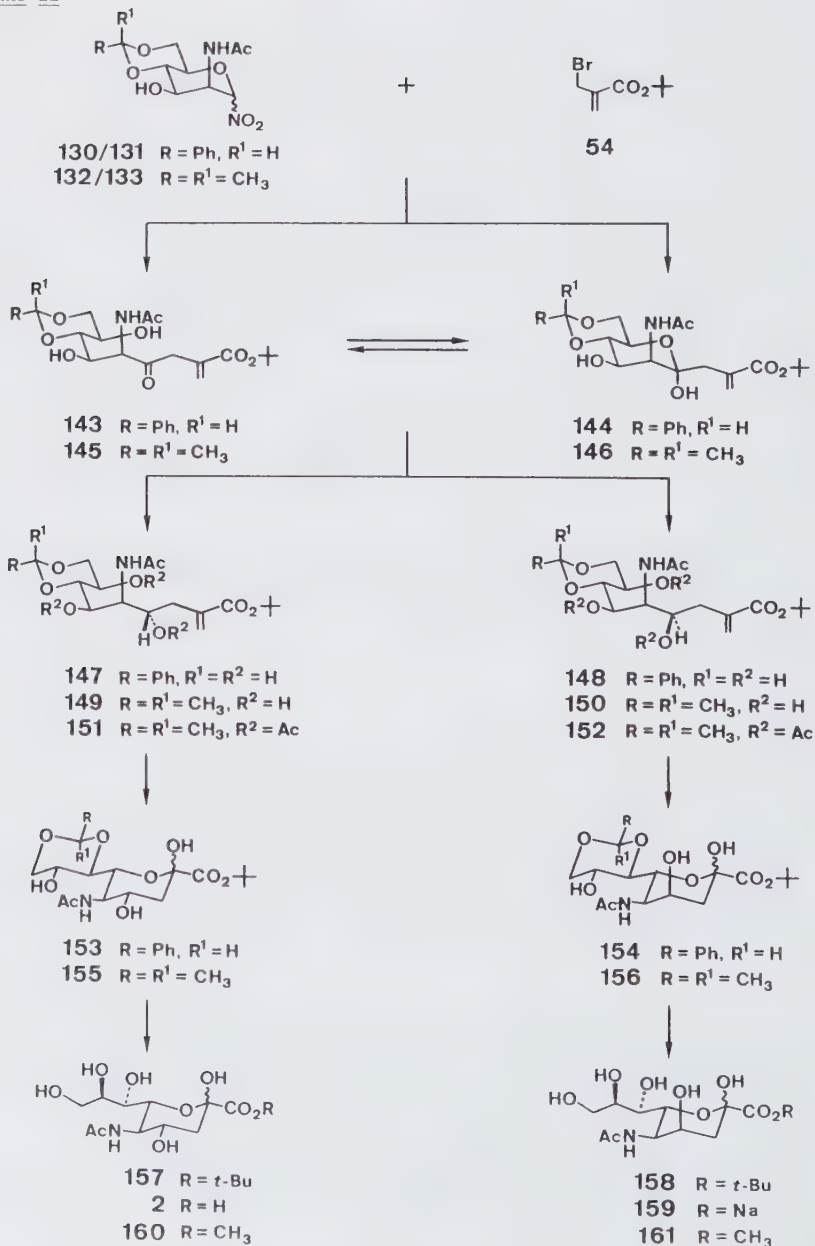
Reduction of the benzyldiene acetals 143/144 and the isopropylidene acetals 145/146 with NaBH_4 in the absence of AcOH always resulted in the D-glycero-D-talo-isomer 148 and 150, respectively, as the main products (147/148 and 149/150 = 15:85). The same ratio of the epimeric triols 147 and

and 132/133 showed $\nu(\text{NO}_2)$ at 1555 cm^{-1} and 1553 cm^{-1} , respectively. The position of these bands indicates the major products to be 141 and 142, respectively [147], implying equatorial attack on the Michael acceptor. While 141 was partially transformed into 143 and 144 during aqueous workup, the isopropylidene derivative 142 proved more stable to hydrolysis.

²⁶⁾ The diastereoselectivity in the reduction of ketones depends upon the proportions of hydroxylic and nonhydroxylic components of the solvent [187]. For the use of sodium acyloxy borohydrides in diastereoselective reductions of cyclic ketones see [188], [189].

²⁷⁾ Initial experiments were performed by Martin Hugener (undergraduate research project).

Scheme 22



148 was obtained by the reduction of 143/144 with NaBH_4 in EtOH (95%) or with $\text{Zn}(\text{BH}_4)_2$ [191] in THF (95% combined yield). The diastereoselectivity of these reductions may be rationalized by assuming a H-bond between the acetamido substituent and the C(4) carbonyl group [171]. In the absence of such a H-bond, the Anh-Felkin model [125] predicts the formation of 147 and 149.

Ozonolysis of the epimeric benzylidene acetals 147 and 148 gave the crystalline hemiacetals 153 and 154, respectively. Both compounds showed no mutarotation in MeOH solution after 5 min. The $^1\text{H-NMR}$ ($(\text{D}_6)\text{DMSO}$) and $^{13}\text{C-NMR}$ spectra (CD_3OD) of the neuraminic acid derivative 153 indicated the presence of only the $\beta\text{-D}$ -anomer. In the $^1\text{H-NMR}$ spectrum ($(\text{D}_6)\text{DMSO}$) of the 4-epi-neuraminic acid derivative 154 also, only the $\beta\text{-D}$ -anomer was detected. The $^{13}\text{C-NMR}$ (CD_3OD) and the $^1\text{H-NMR}$ spectrum (CD_3OD) of freshly dissolved 154 showed, however, signals for both anomers ($\alpha:\beta = 1:3$). Thus, compound 154 anomerized slowly in DMSO, but rapidly in MeOH (cf. [192]).

Ozonolysis of the 92:8 mixture of the epimeric isopropylidene acetals 149 and 150 and flash chromatography of the crude product gave the neuraminic-acid derivative 155 (85%) and its 4-epi analogue 156 (7%). Although 155 and 156 did not crystallize, their $^1\text{H-NMR}$ ($(\text{D}_6)\text{DMSO}$) and their $^{13}\text{C-NMR}$ spectra (CD_3OD) showed only traces of the $\alpha\text{-D}$ -anomers. No signals of open-chain tautomers of 153, 154, 155 or 156 were observed.

The $^1\text{H-NMR}$ spectra of the neuraminic-acid derivatives 153 and 155 showed two large coupling constants (153: $J_{4,5} = 9.5$ and $J_{5,6} = 9.1$; 155: $J_{4,5} = 9.8$ and $J_{5,6} = 9.0$ Hz), characteristic for a 1,2 diaxial orientation of H-C(4)/H-C(5) and H-C(5)/H-C(6). The corresponding coupling constant of the 4-epi derivatives 154 and 156 were $J_{4,5} = 3.0$ (154 and 156) and $J_{5,6} = 10.5$ (154) and 9.0 (156) [88][108], clearly indicating a 1,2 diaxial relation of H-C(5)/H-C(6) and a 1,2-synclinal relation of H-C(4)/H-C(5).

The benzylidene acetal 153 was transformed into Neu5Ac 2 by hydrogenolysis of the benzylidene group (Pd-C , aq. MeOH, AcOH; 94%) and saponification of the resulting crystalline tert-butyl ester 157 (K_2CO_3 , aq. MeOH;

>95% of 2, 51% after crystallization [6][72]). This product could not be distinguished from an authentic sample of N-acetylneuraminic acid (Fluka; m.p., mixed m.p., $[\alpha]_D$ and $^1\text{H-NMR}$ spectrum [72][193]). The structure of 2 was confirmed by preparation of the known methyl ester 160 [193][194]. The tert-butyl ester 157 was also obtained from the isopropylidene acetal 155 by hydrolysis with CF_3COOH in CH_2Cl_2 (85%). It did not mutarotate in H_2O solution. According to the $^1\text{H-NMR}$ spectrum it is a 9:1 mixture of the β -D- and α -D-anomers.

Similarly, sodium N-acetyl-4-epi-neuramate (159) was obtained from the benzylidene acetal 154, which gave the crystalline tert-butyl ester 158 (95%) upon hydrogenolysis. Saponification of 158 and sequential chromatography on Dowex 1 (HCOO^-) and Dowex 50 (Na^+) gave the sodium N-acetyl-4-epineuramate (159) (46%)²⁸) as a 1:9 mixture of the α - and β -D-anomers ($^1\text{H-NMR}$).

Comparison of the $^{13}\text{C-NMR}$ spectrum of 159 ($\text{R}=\text{H}$) with the one of sodium N-acetyl- β -neuramate (2; $\text{R}=\text{Na}$; pD 7) [44][195] shows an upfield shift of 3.9 and 5.4 ppm for C(3) and C(5 and 6) of 159, respectively. The upfield shift of 5.4 ppm for C(6) is a typical value [196] for the difference of the γ -effects exerted by the equatorial and the axial hydroxy group at C(4) of 2 ($\text{R}=\text{Na}$) and 159, respectively.

The tert-butyl ester 158 was also obtained from the isopropylidene acetal 156 by hydrolysis with CF_3COOH in CH_2Cl_2 (65%). It did not mutarotate in H_2O solution after 5 min. Its $^1\text{H-NMR}$ spectrum showed the presence of a 7:3 mixture of the β - and α -D-anomers. The methyl ester 161 was obtained in high yields by treating the tert-butyl ester 158 first with CF_3COOH in chlorobutane and then with CF_3COOH in MeOH.

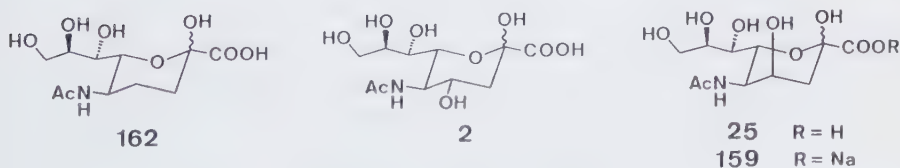
²⁸) The free acid has not been described in detail. It did not crystallize in our hands, but decomposed slowly, forming a red oil. Preliminary results have been published by Brossmer and coworkers [79].

6. Synthesis of N-Acetyl-4-deoxyneuraminic Acid

6.1. Introduction

Although the function of neuraminidases and the effect of sialic acids, their derivatives and their glycosides upon these enzymes have been extensively studied [14][22][180][197][198], little is known about the detailed mechanism of their action. Schauer et al. [90][180][181] have shown that glycosides of N,4-O-diacetylneuraminic acid and N-acetyl-4-O-methylneuraminic acid were strongly resistant to mammalian and bacterial sialidases, and Flashner et al. [99][178][179][199] have shown that N-acetyl-2-deoxy-4-epineuraminic acid, N-acetyl-2,3-dehydro-4-epineuraminic acid and N-acetyl-2,3-dehydro-4-oxoneuraminic acid are competitive inhibitors of *Arthrobacter sialophilus* neuraminidase and of influenza virus neuraminidase. These results indicate an important role for the C(4) OH-group.

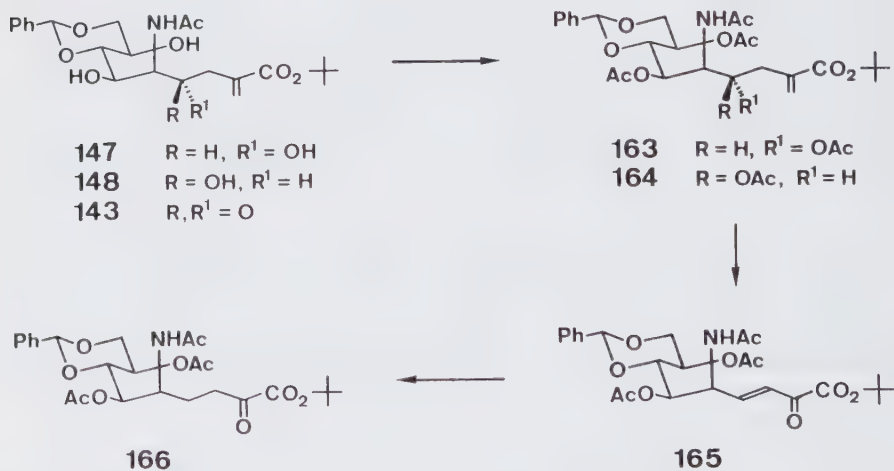
We, therefore, wished to prepare the N-acetyl-4-deoxyneuraminic acid (4-deoxy-Neu5Ac, **162**) exploiting our synthesis of N-acetylneuraminic acid (Neu5Ac, **2**) and N-acetyl-4-epineuraminic acid (4-epi-Neu5Ac, **159**) (see Chap. 5). Intermediates of this synthesis, which appear to be suitable candidates for a deoxygenation at C(4) are the epimeric alcohols **147** and **148** (Scheme 23).



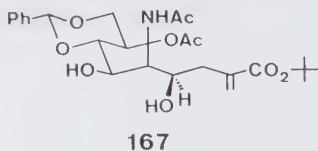
6.2. Results

Acetylation (Ac_2O /pyridine 1:2) of a crude 1:3 mixture of the triols **147** and **148** (see Chap. 5), obtained by NaBH_4 reduction of the ketone **143**, gave the tetra-acetates **163** and **164**²⁹ (Scheme 23). Pure samples of the tetra-acetates **163** and **164** were obtained by chromatography and also by acetylation of the individual triols **147** and **148**.

Scheme 23



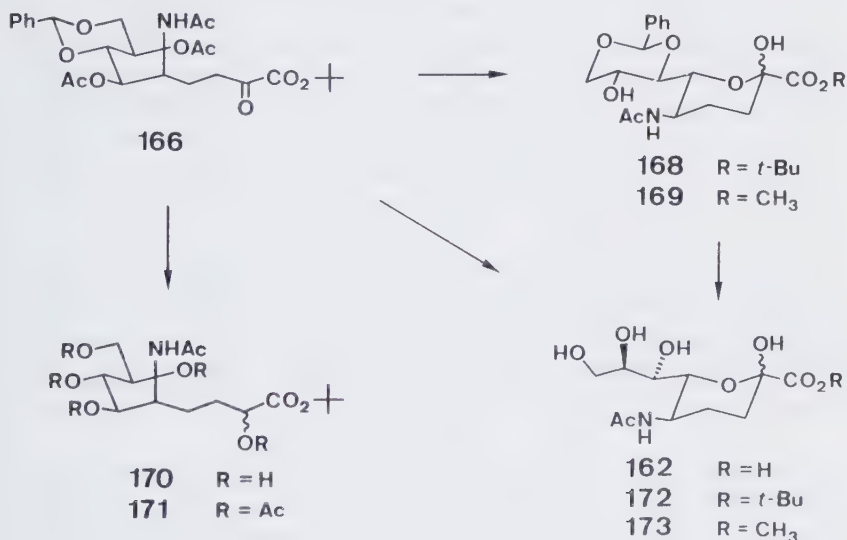
²⁹) Besides **163** and **164**, we obtained 10-20% of the mono-O-acetate **167**, which was not converted to the tetra-acetate **164** even upon addition of a



large excess of Ac_2O /pyridine or in the presence of 4-(dimethylamino)-pyridine (formation of borate esters?), while acetylation (Ac_2O /pyridine) of a pure sample of **167** gave the tetra-acetate **164** under mild conditions.

Ozonolysis of a mixture of the tetra-acetates **163** and **164** in CHCl_3 at -60° and in the presence of NaHCO_3 followed by addition of $(i\text{-Pr})_2\text{EtN}$ gave the (E)-configured α,β -unsaturated keto ester **165**. Its IR spectrum is characterised by absorptions at 1704 cm^{-1} (C=O), 1678 cm^{-1} (NHAc), and at 1630 cm^{-1} (C=C), and its $^1\text{H-NMR}$ spectrum by $J_{3,4} = 16\text{ Hz}$. Hydrogenation of **165** (10% Pd/C , AcOEt) gave the saturated keto ester **166**³⁰.

Scheme 24



³⁰) Hydrogenolysis of the benzyldiene group has not been observed under these conditions. Two-dimensional TLC of **166** revealed the presence of (at least) three interconverting compounds. The $^1\text{H-NMR}$ spectrum showed 4 signals for the tert-butyl group and two NH signals. These observations may be rationalized by assuming the presence of two anomeric 'furanoses' [200], the ketone **166** and the enol corresponding to it (none of the products described in this or the preceding chap. showed multiple signals of the NHAc group due to rotational isomerism).

Saponification of the keto ester 166 with K_2CO_3 in aq. MeOH (Scheme 24) followed by acid hydrolysis (Dowex 50 (H^+), aq. dioxane), and purification of the resulting crude N-acetyl-4-deoxyneuraminic acid (162) by anion exchange chromatography (Dowex 1 ($HCOO^-$)) led to 162 as a microcrystalline solid (53% from 163/164).

The 1H -NMR spectrum of 162 showed only signals of the β -D-anomer. Comparison of the 1H -NMR spectrum of 162 with the one of N-acetylneuraminic acid (2) [193] showed similar chemical shifts for H-C(6) to $CH_2(9)$ ($\Delta\delta \pm 0.1$ ppm) and the same coupling constants with the exception of $J_{6,7}$, where a difference of 1 Hz is noted. The signal of H-C(5) in the spectrum of 162 is shifted downfield and overlaps the one of H-C(6) at 4.13 - 3.93 ppm. A similar comparison of the ^{13}C -NMR spectra (see [44]) shows an upfield shift of 0.5-1.0 ppm for the signals of C(7), C(8), and C(9) of 162, one of 8.2 and 8.8 ppm, respectively, for the signals of C(5) and C(3), and a downfield shift of 0.7 ppm for the one of C(6).

De-O-acetylation of the keto ester 166 with NaOMe/MeOH at 0° gave, after flash chromatography, the crystalline tert-butyl ester 168 and the crystalline methyl ester 169 (54% from 7/8; Scheme 24). Both compounds did not mutarotate in MeOH solution, and their 1H -NMR and ^{13}C -NMR spectra ($(D_6)DMSO$) showed signals of the β -D-anomer only.

A comparison of the 1H -NMR spectra of the N-acetyl-4-deoxyneuraminic-acid derivative 168 with the one of the corresponding N-acetylneuraminic-acid derivative (see Chap. 5) showed approximately the same chemical shifts ($\Delta\delta \pm 0.01$ ppm) and the same coupling constants for the H-atoms at C(7), C(8), and C(9), while the signals of H-C(6) and H-(5) were slightly shifted to lower fields (0.05 ppm) and $J_{5,6}$ and $J_{6,7}$ were larger by 1 Hz for the N-acetyl-4-deoxyneuraminic-acid derivative 168. A similar comparison of the ^{13}C -NMR spectra showed an upfield shift of 1-2 ppm for C(6), C(7), C(8), and C(9) and one of ca. 10 ppm for C(3) and C(5) for 168.

Hydrogenolytic removal of the benzylidene group of 168 and 169, respectively, gave the crystalline tert-butyl N-acetyl-4-deoxyneuraminate (172) and the crystalline methyl N-acetyl-4-deoxyneuraminate (173), which was also obtained by treating 162 with CH_2N_2 or CF_3COOH in MeOH.

Hydrogenolysis of the keto ester 166 in the presence of 20% $\text{Pd}(\text{OH})_2$ (MeOH) [201] followed by de-O-acetylation of the intermediate (K_2CO_3 in anhyd. MeOH) gave the overreduction products 170 (68% from 163 and 164) instead of the desired 172. Acetylation of 170 gave a mixture of the acetates 171, which were characterized by the presence of 5 O-acetyl groups in their ^1H -NMR spectrum. The ^{13}C -NMR spectrum of 171 showed an additional doublet at ca. 70 ppm, but no carbonyl resonance at ca. 210 ppm. The NMR spectra showed the presence of two diastereoisomers in a ratio of about 2:3.

7. 4-Methylumbelliferyl 5-Acetamido-3,4,5-trideoxy- α -D-manno-2-nonulopyranosidonic Acid: Synthesis and Resistance to Bacterial Sialidases³¹⁾

7.1. Introduction

The C(4)-OH group of sialic acids seems to be important for the cleavage of sialic-acid glycosides by sialidases as summarized in Chap. 6 (cf. also [14]). Sialic acids acetylated at C(4) are not released by sialidases. To elucidate, whether the bulkiness of a substituent, e.g. an AcO group, at C(4) or the absence of the free OH group is responsible for the resistance of the glycosidic linkage towards sialidase action, we wanted to test the cleavage of an appropriate α -D-glycoside of N-acetyl-4-deoxyneuraminic acid (162) by sialidases. Among the glycosidase-assay methods based upon the quantification of the liberated aglycone [202-204], the method of Thomas et al. [205], using glycosides of 4-methylumbelliferone, has the advantage of allowing a fluorometric evaluation of the liberated aglycone. The higher sensitivity of fluorometric over colorimetric substrates for glycosidase assays has been established by several authors [206-209]. We, therefore, prepared the 4-methylumbelliferyl α -D-glycoside 181 of N-acetyl-4-deoxyneuraminic acid to test it in bacterial sialidase activity assays.

7.2. Synthesis of the 4-Methylumbelliferyl α -D-Glycoside of N-Acetyl-4-deoxyneuraminic Acid

Treatment of N-acetyl-4-deoxyneuraminic acid (4-deoxy-Neu5Ac, 162) with CF₃COOH in anh. MeOH gave the methyl ester 173 (see Chapt. 6). Applying a procedure similar to the one described by Meindl and Tuppy [210], crude 173 was converted into the anomeric peracetates 174 and 175 in a ratio of 3:7 (89% combined yield from 162, see Scheme 24) and into the

³¹⁾ The biochemical part has been investigated by R. Schauer and coworkers, Universität Kiel.

open-chain enol acetate 176 (6% from 162).

The configuration at the anomeric centre of the peracetates 174 and 175 was assigned by comparison of their molecular optical rotation with the one of 4-deoxy-Neu5Ac (162) and of N-acetyl-neuraminic acid (Neu5Ac, 2) on the one hand, and with the one of the known peracetylated methyl neuramate 39 [194] on the other hand (see Table 1). The major acetylation product (62.5%) of methyl N-acetyl-4-deoxyneuramate (173) showed a negative molecular optical rotation at 589 nm ($[M]_D = -68.5^\circ$), the minor product (26.5%) a positive one ($[M]_D = +228.5^\circ$). Both Neu5Ac (2) and 4-deoxy-Neu5Ac (162) showed negative $[M]_D$ -values. The known peracetylated methyl ester 39 shows also a negative $[M]_D$ -value (-17.6°). Hence, we attributed the β -D-configuration to the isomer with the negative $[M]_D$ -value, i.e. (175), and the α -D-configuration to the isomer with the positive $[M]_D$ -value, i.e. (174).

Scheme 25

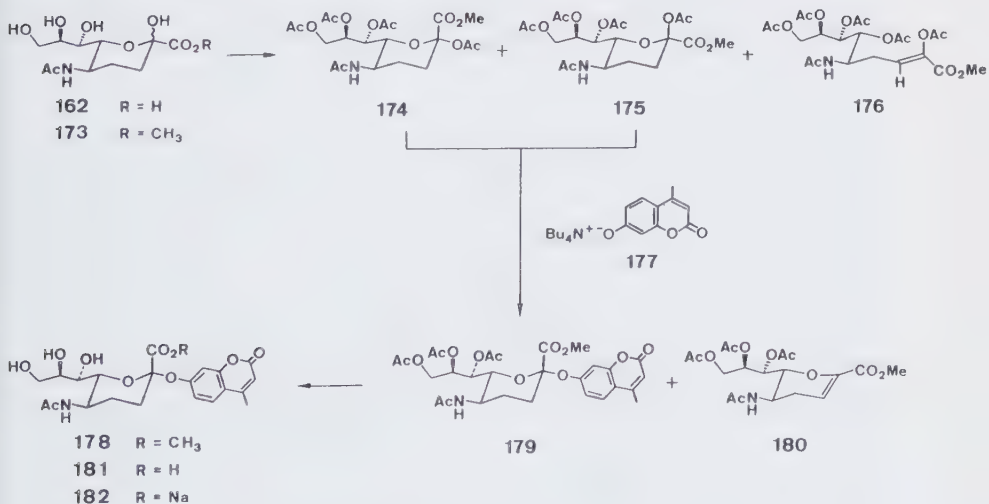
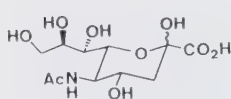
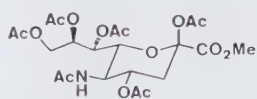


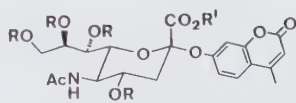
Table 1: Molecular Optical Rotations of Sialic-Acid Derivatives



2



39



184 R = Ac, R' = CH₃

185 R = H, R' = H

Compound	Molecular weight	$[\alpha]_D$ (solv.)	$[M]_D$	$\Delta[M]_D$	reference
<u>163</u>	293.3	-47.6° (H ₂ O)	-139.6°		
<u>2</u>	309.3	-32.1° (H ₂ O)	-99.3°	$\Delta = -40.3^\circ$	[72]
<u>175</u>	475.5	-14.4° (CHCl ₃)	-68.5°	$\Delta_1 = -50.9^\circ$	
<u>174</u>	475.5	+48.1° (CHCl ₃)	+228.7°	$\Delta_2 = +246.3^\circ$	
<u>39</u>	533.5	-3.3° (CHCl ₃)	-17.6°		[194]
<u>179</u>	591.6	+64.8° (CHCl ₃)	+383.4°	$\Delta = +45.6^\circ$	
<u>184</u>	649.6	+52.0° (CHCl ₃)	+337.8°		[215]
<u>178</u>	465.5	+59.4° (CHCl ₃)	+276.5°		
<u>181</u>	451.5	+79.3° (H ₂ O)	+358.0°	$\Delta = +30.7^\circ$	
<u>185</u>	467.5	+70.0° (H ₂ O)	+327.3°		[215]

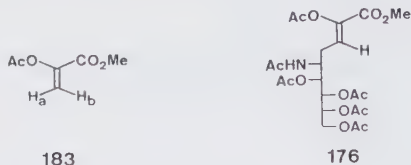
a) $\Delta_1 = [M]_D(\text{175}) - [M]_D(\text{39})$;
b) $\Delta_2 = [M]_D(\text{174}) - [M]_D(\text{39})$.

Due to the interaction of the axial H-C(6) with an axial acetoxy group (see 175) or an axial methoxycarbonyl group (see 174) one should expect a chemical shift difference for H-C(6) in 174 and 175. We observed the signal of H-C(6) at 4.71 ppm in the ^1H -NMR spectrum of the α -D-anomer 174, and at about 4.0 ppm (overlapping the signal of H-C(5)) in the one of the β -D-anomer 175.

The enol acetate 176 is characterized by an UV-absorption at 213 nm indicating an α,β -unsat. ester carrying an acetoxy substituent at C(α) [211]. Signals of an olefinic proton at 6.56 ppm and of 5 O-acetyl groups between 2.27-1.91 ppm in the ^1H -NMR spectrum of 176, as well as a singlet for C(2) (140.4 ppm) and a doublet for C(3) (126.8 ppm) in its ^{13}C -NMR spectrum confirm the constitution of the enol acetate 176. The (Z)-configuration of 176 was deduced from the (C(1),H-C(3))-coupling constant (see Table 2). This coupling constant is significantly different for the (E) and (Z) configuration. A cis-coupling is characterized by smaller values (usually 3-8 Hz) than a trans-coupling (10-18 Hz) [212][213]. To simplify the spectrum we irradiated the proton signal of the methoxy group. This causes a reduction of the J_{CH} -coupling constants, the extent of which depends on $\Delta\delta$ between the irradiated protons and the coupling partners of the observed ^{13}C -NMR signal. To determine the reduction factor we measured the J_{CH} couplings of methyl 2-acetoxy-2-propenoate (183) [214] in the fully coupled and the selectively decoupled ^{13}C -NMR spectrum, irradiating with the same power distribution as used in the case of 176. The coupling constants with H_a were reduced by a factor of ca. 0.84 ($\Delta\delta = 1.68$ ppm), those with H_b by a factor of ca. 0.92 ($\Delta\delta = 2.24$ ppm). In the enol acetate 176, $\Delta\delta$ between H-C(3) and the methoxy group is 2.78 ppm. The reduction factor for 176 should, therefore, be somewhat larger than the corresponding ones for 183. The observed $^3J_{\text{C,H}}$ -coupling of 3.5 Hz in the selectively decoupled spectra of 176 should correspond to ca. 4 Hz in the fully coupled spectrum, which is too small for a trans-coupling. Hence, the enol acetate 176 must be (Z)-configured³²).

³²) We thank Dr. R. Kunz for recording the spectra and for helpful discussions.

Table 2: C,H-Coupling Constants [Hz] of **176** and **183**



coupling constant	183			176	
	fully coupled [Hz]	sel. decoupled [Hz]	red. factor	sel. decoupled [Hz]	
$^1J_{CH}$	163 Hz	138 Hz	0.85	$^1J = 152 \text{ Hz}$	
$^1J_{CH}$	166 Hz	150 Hz	0.95		
$^2J_{CH}$	6.4 Hz	5.5 Hz	0.86	$^2J = 4.5 \text{ Hz}$	
$^2J_{CH}$	3.6 Hz	3.3 Hz	0.92		
$^3J_{CH}$	10.0 Hz	8.2 Hz	0.82	$^3J = 3.5 \text{ Hz}$	
$^3J_{CH}$	3.5 Hz	3.1 Hz	0.89		
$^3J_{CH}$	2.8 Hz				

A mixture of the anomeric peracetylated methyl esters **174** and **175** was transformed into the glycosyl chloride (HCl, AcCl, ether) employing a similar procedure as described by Kuhn et al. [194] for the preparation of the methyl acetochloroneuraminatate (Scheme 25). The resulting glycosyl chloride was too unstable to be isolated, and therefore was directly converted into the 4-methylumbelliferyl ketoside **179** by treatment with the tetrabutylammo-

nium salt 177 of 4-methylumbelliferone [215] in the presence of freshly prepared Ag_2CO_3 (Scheme). Chromatographic purification gave the ketoside 179³³) (75% from 174/175) and the 2,3-dehydro-4-deoxy-neuraminic acid derivative 180 (17% from 174/175). As shown by TLC, 180 was already present before the glycosyl chloride was subjected to the Königs-Knorr glycosidation [216], where formation of 2,3-dehydro sialic acids is well known [94].

The 4-methylumbelliferyl ketoside 179 was deprotected according to Zemlen, as modified by Baggett and Marsden [215] to give the de-O-acetylated glycoside 178 in 95% yield. The methyl ester was saponified with aq. NaOH to give, after chromatography, 4-methylumbelliferyl α -D-glycoside 181 (MU-4-deoxy-Neu5Ac) of N-acetyl-4-deoxyneuraminic acid in 78% from 179. The free acid 181 was quite unstable and decomposed progressively, presumably by self-catalyzed hydrolysis [208]. It was, therefore, transformed into the sodium salt 182 by passage through Dowex 50 (Na^+). Esterification of the free acid 181 with CH_2N_2 in MeOH solution gave again the methyl ester 178. Except for the OH-signals, the IR and $^1\text{H-NMR}$ spectra of 178 obtained in this way were indistinguishable from those obtained by de-O-acetylation of 179 as detailed above.

7.3. Determination of the Configuration at C(2) of the Compounds 178, 179, 181, and 182.

The molecular optical rotations of N-acetyl-4-deoxyneuraminic acid (162) and its derivatives on the one hand and of N-acetylneuraminic acid (2) and its derivatives on the other hand are collected in Table 1. The acid 162 and its fully acetylated ester 175 (major acetylation product) possess negative $[\text{M}]_{\text{D}}$ -values like Neu5Ac (2) and the fully acetylated methyl neuraminate 39. The difference between the $[\text{M}]_{\text{D}}$ -values of 162 and 2 (-40.3°) and of

33) No other product containing a 4-methylumbelliferyl group was observed (cf. [208]).

175 and 39 (-50.9°) can be rationalized by an additional 'gauche' ((+)-sc) interaction of the C(4) OH group with the C(5) NHAc group in the neuraminic-acid derivatives, which is absent in the 4-deoxyneuraminic acid and its derivatives [217][218]. Inversion of the configuration at C(2) (cf. 174, 184, 185) led to positive $[M]_D$ -values. We found positive molecular optical rotations also for the 4-methylumbelliferyl ketosides 178, 179, 181, and 182 and considered this as an indication for the α -D-configuration of these ketosides.

Comparison of the ^{13}C -NMR spectrum of the 4-methylumbelliferyl α -D-glycoside 181 of N¹-acetyl-4-deoxyneuraminic acid with the one of the corresponding 4-hydroxy derivative 185 [215] showed similar chemical shifts for all C-atoms with the exception of those for C(3) to C(6). The signal of C(6) in 181 is shifted downfield (2.2 ppm, γ -effect, [196]; see also Chap. 6), the signals of C(3)-C(5) are shifted upfield (cf. Chap. 6). This similarity in the ^{13}C -chemical shifts of 181 and 185 constitutes another evidence for the α -D-configuration of the ketoside 181.

The values of the chemical shift of C(1) of the 4-deoxy-Neu5Ac ketoside 181 (173.8 ppm) and its methyl ester 178 (169.1 ppm) agree well with the corresponding ones of the α -D-methyl ketoside of Neu5Ac 2 (174.3 ppm) and its methyl ester (170.7 ppm) published by Brossmer and Eschenfelder [219]; the C(1) signals for the corresponding β -D-ketosides were found at 176.1 ppm (acid) and 171.2 ppm (ester) [219].

A further evidence for the α -D-configuration of neuraminic acid ketosides is found in the chemical shift of $\text{H}_{\text{eq}}\text{-C}(3)$. Brossmer and coworkers [220] have shown that signals of $\text{H}_{\text{eq}}\text{-C}(3)$ of α -D-ketosides of Neu5Ac 2 (D_2O soln.) appear between 2.60 and 2.75 ppm, and those of $\text{H}_{\text{eq}}\text{-C}(3)$ of the corresponding β -D-ketosides between 2.25 and 2.40 ppm. We found $\text{H}_{\text{eq}}\text{-C}(3)$ at 2.54 ppm (181) and 2.55 ppm (182), respectively. The slight upfield shift in the 4-deoxyneuraminic-acid derivatives may be rationalized by the absence of the OH group at C(4) (absence of a β -effect).

7.4. Sialidase (EC 3.2.1.18) Experiments.

The MU-4-deoxy-Neu5Ac 181 was tested in sialidase-activity assays with sialidases from Vibrio cholerae and Arthrobacter ureafaciens (see Exper. Part). In addition, the influence of free 4-deoxy-Neu5Ac 162 and of MU-4-deoxy-Neu5Ac 181 on sialidase activity was checked with the 4-methylumbelliferyl α -D-glycoside 185 (MU-Neu5Ac) of N-acetylneuraminic acid as substrate.

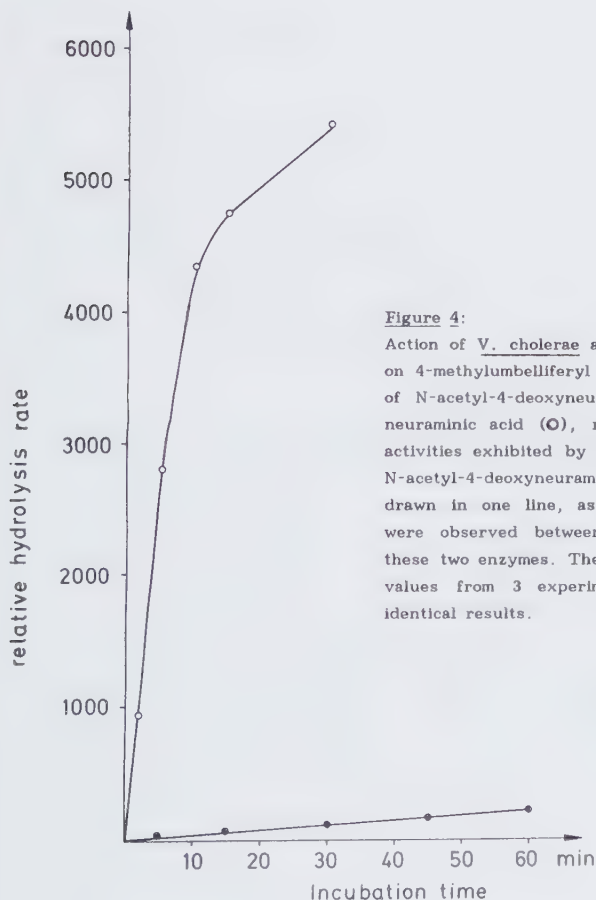


Figure 4:

Action of V. cholerae and A. ureafaciens sialidases on 4-methylumbelliferyl α -D-glycosides 181 and 185 of N-acetyl-4-deoxyneuraminic (●) and N-acetylneuraminic acid (O), respectively. The very low activities exhibited by the two sialidases with the N-acetyl-4-deoxyneuraminic acid glycoside are drawn in one line, as no significant differences were observed between the hydrolysis rates of these two enzymes. The values in Fig. 4 are mean values from 3 experiments, which gave almost identical results.

The MU-4-deoxy-Neu5Ac 181 is hydrolyzed by the bacterial sialidases at extremely low rates. When compared with the initial hydrolysis rate of the corresponding 4-hydroxy derivative 185, MU-4-deoxy-Neu5Ac 181 is hydrolyzed under the influence of both sialidases only by 1.4% of this rate (Fig. 4).

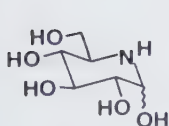
Both free 4-deoxy-Neu5Ac 162 and its 4-methylumbelliferyl α -D-glycoside 181 do not inhibit sialidase activity at the concentrations applied, as was tested with MU-Neu5Ac 185 as substrate.

These experiments demonstrate the requirement of an OH group at C(4) of the neuraminic acid molecule for hydrolysis of the α -glycosidic bond. Absence of this residue seems to prevent binding of sialic acid to the active center of the sialidases tested, as no (competitive) inhibition of the hydrolysis of MU-Neu5Ac 185, an excellent substrate for sialidases (Fig.), could be observed neither with free 4-deoxy-Neu5Ac 162 nor its α -D-glycoside 181. In contrast, free Neu5Ac 2 with an equatorial hydroxyl group at C(4) is a competitive inhibitor of sialidase at the concentrations used here [14]. Substitution of the OH group at C(4) by an AcO group [14][221] prevents hydrolysis of the glycosidic groups of sialic acids by bacterial sialidases, too. In addition, methylation of the C(4) hydroxyl group [90] strongly reduces the hydrolysis rate of this sialic acid by *V. cholerae* sialidase. These residues also seem to prevent or hinder binding of sialic acid to the active center of sialidase, as 4-O,N-diacetylneuraminic acid does not competitively inhibit sialidase action, similar to the 4-deoxy-Neu5Ac 162 tested here. Epimerization of the OH group at C(4) in neuraminic acid into axial position to give N-acetyl-4-epineuraminic acid also reduces the affinity to sialidases. Particularly, we could not observe inhibition of the bacterial sialidases by this neuraminic acid isomer at millimolar concentration. It would be tempting to investigate more closely the role of the equatorial C(4) OH group of neuraminic acid in the mechanism of sialidase catalysis.

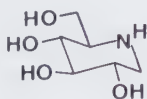
8. Synthesis of 6-Amino-6-deoxy-sialic Acids

8.1. Introduction

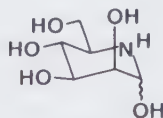
Naturally occurring enzyme inhibitors are frequently found in plants, fungi, bacteria and animals. The antibiotic nojirimycin (**186**) [222][223] and its 1-deoxy-derivative **187** [200][224] inhibit α - and β -D-glucosidases up to 10^5 times better than D-glucose [225]. Both compounds have been isolated from the culture broth of streptomyces [222][224]. Nojirimycin B **188**, isolated from, '*Streptomyces Lavendulae*' [226], 1-deoxy-mannojojirimycin **189** [227][228] and the indolizidine alkaloid swainsonine (**190**) [229][230] both of plant origin, inhibit the action of mannosidases up to 10^2 - 10^4 times stronger than D-mannose [231][232]. Whilst the naturally occurring amino acids 2S-Carboxy-3R,4R,5S-trihydroxypiperidine (**191**) [233 - 235] (isolated from the seeds of the legume *Baphia racemosa* [233][234]) and 3R,4R-dihydroxy-L-proline (**192**) [236][237] of fungal origin *Amanita virosa* [236]) inhibit β -D-glucuronidases, Siastatin, for which the somewhat surprising structure **193** has been proposed, is a sialidase inhibitor. Siastatin **193** was isolated from *Streptomyces verticillus* [238][239].



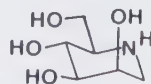
186



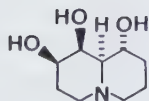
187



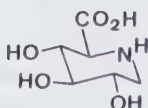
188



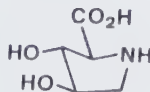
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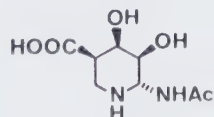
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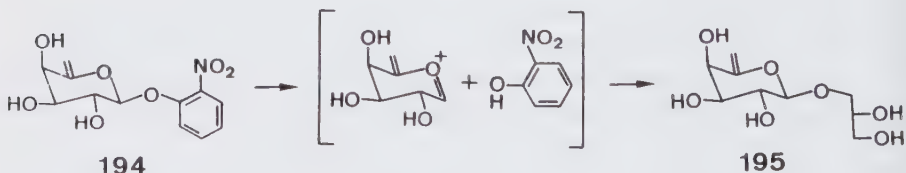


193

It has been found that the inhibition of glycosidase activity by these piperidine compounds is competitive and reversible [240-242]. It was also demonstrated that the inhibition of the enzymatic activity by these compounds is pH-dependent, and that the unprotonated amine is the effective inhibitor [243][244].

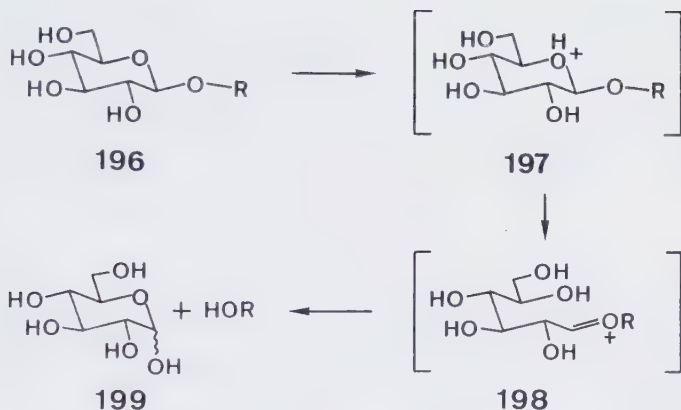
In mechanistic studies, Lehmann and Reinshagen [245] have demonstrated that in the enzymatic hydrolysis of the *o*-nitro-phenylglycoside **194** (Scheme 26), which is a substrate of β -galactosidase, protonation of the exocyclic oxygen must be the initial step. In the presence of glycerol as acceptor, the β -D-glycerol-pyranoside **195** was formed, requiring the transfer of the sugar component as a cyclic unit.

Scheme 26



Fleet [240], however, postulates the protonation of the ring oxygen to be the initial step. He suggests that hydrolysis of α - and β -glycosides such as **196**, is initiated by protonation of the endocyclic oxygen to give **197** (Scheme 27). Subsequent cleavage of the C(1)-O(5)-bond gives **198**. Attack of **198** by water, fission of the C(1)-OR bond and ring closure leads finally to **199** and to the aglycone HOR. This mechanism was proposed to rationalize the glycosidase inhibition by the protonated piperidines **186** - **193**, which are close analogues of **197**. Post and Karplus [246] have presented a similar mechanism based on molecular dynamics simulation of the enzymatic hydrolysis of an oligosaccharide.

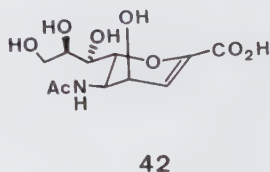
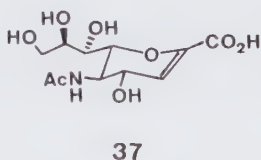
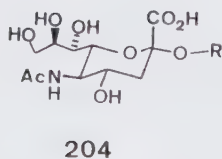
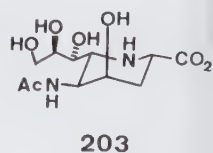
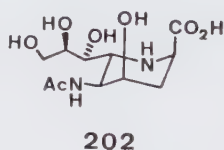
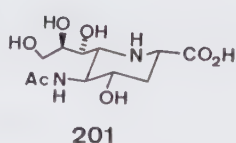
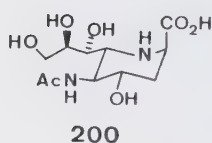
Scheme 27



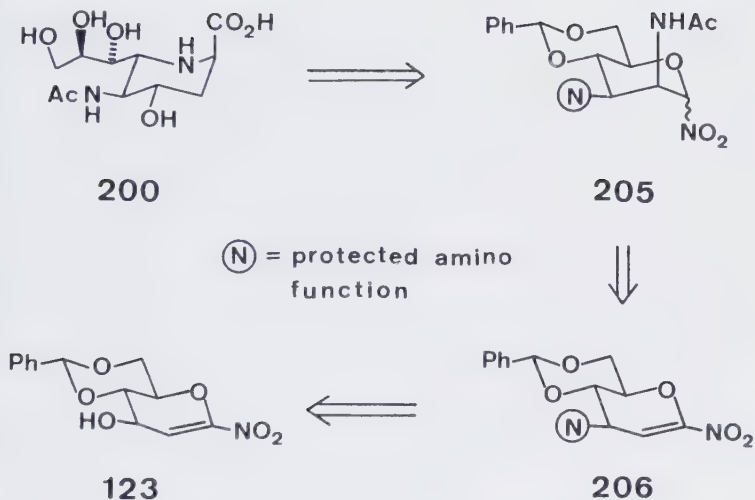
Sialidases are the most important enzymes for the initiation of the catabolism of sialo-oligosaccharides and sialoglycoconjugates. Sialidases may have toxic effects when present in high, nonphysiological amounts. Absence of the enzyme or its presence in insufficient quantities also leads to pathological consequences, such as some forms of mucopolidoses and sialidoses [197] [247-248].

In myxoviruses, the sialidase and a second enzyme, haemagglutinin, are localized on the outer-envelope of the virion particle. The viral sialidase plays a decisive role in the process of virus multiplication [249] [250]. In infected cells, Neu5Ac residues from both viral and cellular glycoproteins and glycolipids have been removed. As a consequence, the membranes of the infected cells no longer contain receptors for the viral haemagglutinin and the release of virus particles can proceed [50]. Failure of this receptor destroying mechanism in cells leads to the binding and accumulation of virus particles at the cell surface [198]. Inhibition of sialidase action is therefore a target for antiviral drugs [251].

For this reason and considering the general importance of specific inhibitors we planned to synthesize a sialidase inhibitor. By analogy with the inhibitory effects of the piperidine derivatives 186-193 the amino acid 200 should be the ideal inhibitor for sialidases cleaving α -D-glycosides 204 of N-acetylneuraminic acid. Since 2,3-dehydro-N-acetylneuraminic acid 37 [99][252-254] and N-acetyl-2,3-dehydro-4-epi-neuraminic acid 42 [99] also inhibit sialidase activity, the amino acids 201-203 are also compounds of interest.



Scheme 28

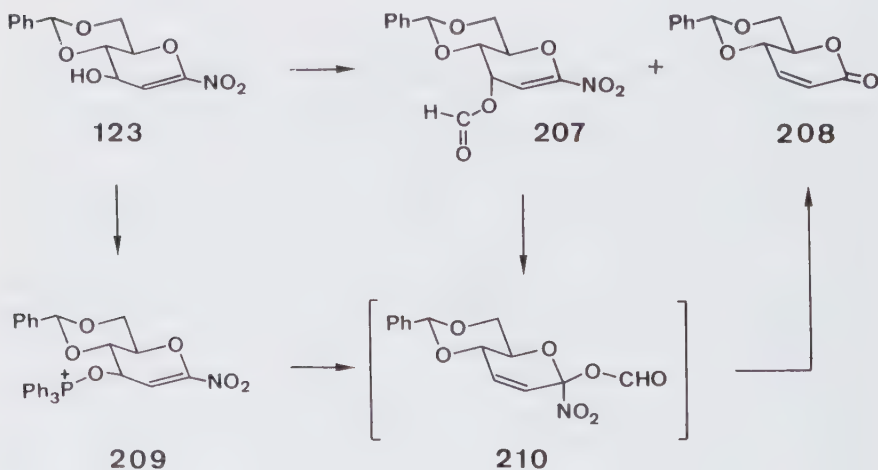


To take advantage of our synthesis of Neu5Ac and 4-epi-Neu5Ac (see Chap. 5) we had to prepare a N-acetylmannosamine derivative, such as **205**, containing a nitrogen function at C(3) (corresponding to C(6) of Neu5Ac; Scheme 28). This approach requires the preparation of a hexose with two different nitrogen functions at C(2) and C(3). We hoped to obtain the desired sugar from the nitro olefin **123**, by introducing first a nitrogen function at C(3) by double inversion ($\rightarrow$ **206**), and then an acetamido group at C(2) by 1,4-addition of NH₃ and acetylation giving the manno configured nitro sugar **205**.

8.2. Results

To introduce a nitrogen function at C(3) of 123 (corresponding to C(6) of Neu5Ac), the C(3)-OH group of the nitro olefin 123 either had to be substituted by a nitrogen-containing functional group under retention of the configuration, or under double inversion, the latter possibility appearing more feasible. To a solution of the nitro olefin 123 and PPh_3 in THF at 5° was added a mixture of diethylazodicarboxylate (DEAD) and formic acid in THF over 48 h to give after chromatography the *D*-ribo-configured nitro olefin 207 (56%) and the known δ -lactone 208 [255] (27%, Scheme 29). Shorter reaction times and/or higher reaction temperatures as well as the addition of the nitro olefin 123 to the mixture of the reagents [256] led to increased amounts of the by-product 208.

Scheme 29



The ^1H -NMR spectra of the nitro olefin 207 differs from those of the C(3)-epimer 127 (see Chap. 4) only by a larger $J_{2,3^-}$ (6.0 Hz for 207, 2.7 Hz for 127) and a smaller $J_{3,4}$ -coupling constant (4.0 Hz for 207, 8.0 Hz for 127), indicating an inversion of the configuration at C(3).

The δ -lactone 208 may be formed during work up from the hypothetical intermediate 210. This intermediate could derive from either 207 or 209 by an S_N2' or by an S_N1 mechanism according to Scheme 29. Solvolytic removal of the nitro group and elimination of HCOOH from the intermediate hemi-orthoester gives the δ -lactone 208. Dyong et al. [257] have shown that in Mitsunobu reactions [258] of allylic substrates, where the leaving group and an adjacent alkoxy group are in a 1,2-trans relationship, the S_N1' substitution may become the main reaction.

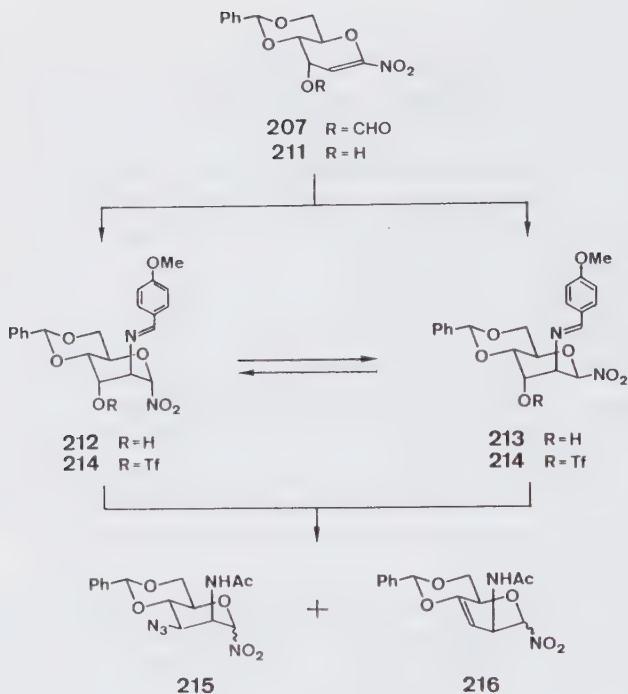
A THF solution of 207 was treated with aq. NH_3 at 0° until it was completely transformed into the alcohol 211, whereupon the reaction mixture was warmed to r.t. This led to the 1,4-addition of NH_3 giving the 1-deoxy-1-nitro-D-altrosamine, which was directly converted into the imino-sugars 212/213³⁴) (89% from 207). Pure 212 was obtained by crystallisation. Equilibration of the imine 212 with NEt_3 in anh. THF at r.t. gave after 10 d a 85:15 mixture (1H -NMR) of 212 and 213 (Scheme 30).

The imine 212 is characterized by an UV-absorption at 283 nm (18'810) typical [259] for the presence of the N-methoxybenzylidene group and an IR-absorption at $1632cm^{-1}$ for a conjugated C=N bond. The D-alto-configuration of the imines 212 and 213, respectively, is deduced from the 3J -coupling constants in their 1H -NMR spectra ($J_{1,2} = 0$ Hz for 212, $J_{1,2} = 2.0$ Hz for 213, $J_{2,3} = 4.0$ Hz for 212 and $J_{3,4} = 2.1$ Hz for 212). Comparison of $J_{1,2}$ of 212 and 213 with the corresponding coupling constants of D-manno- and D-alto-configured 1-deoxy-1-nitro-sugars [147][260] confirm the postulated α -D-configuration of 212.

The alcohol 211 was also obtained in a yield of 79% by treating the formate 210 with NaOMe/MeOH in THF/MeOH 5:2 (0° , 10 min; $J_{2,3} = 5.8$ Hz, $J_{3,4} = 3.8$ Hz).

34) The N-methoxybenzylidene group is a non neighbouring active amino protective group.

Scheme 30



The imine **212** was best transformed into the azide **215** without isolation of any intermediate. Treatment of **212** with trifluoromethanesulfonic acid anhydride in CH_2Cl_2 /pyridine between -30° to 0° gave the anomeric triflate **214** ($\alpha : \beta = 4:1$; 92%; Scheme 30). Attempts to mesylate (MsCl , NEt_3 , 0° , CH_3CN) the imine **212** failed. The triflate group in **214** was substituted with the help of LiN_3 in benzene/HMPT at r.t. to give an azide [261][262]. Similar results were obtained from the reaction of **214** with $\text{Bu}_3\text{PCl}_6\text{H}_{34}\text{N}_3$ in Et_2O at r.t. This method has the disadvantage that the excess reagent must be removed by chromatography [263]. Deprotection of the amino function at C(2) with tosylhydrazide/ AcOH (0° , 20 h) [264] and acetylation ($\text{Ac}_2\text{O}/\text{EtOH}$) gave the 2-acetamido-3-azido-nitrosugar **215** (81% from **212**) and the olefin **216** (13% from **215**).

Neither the olefin 211 nor the imine 212 reacted under conditions of Mitsunobu reaction to give the corresponding azide [265]. Treatment of 211 with $\text{CF}_3\text{SO}_3\text{N}_3$ [266] to cause direct substitution of the C(3)-OH group gave complex mixtures.

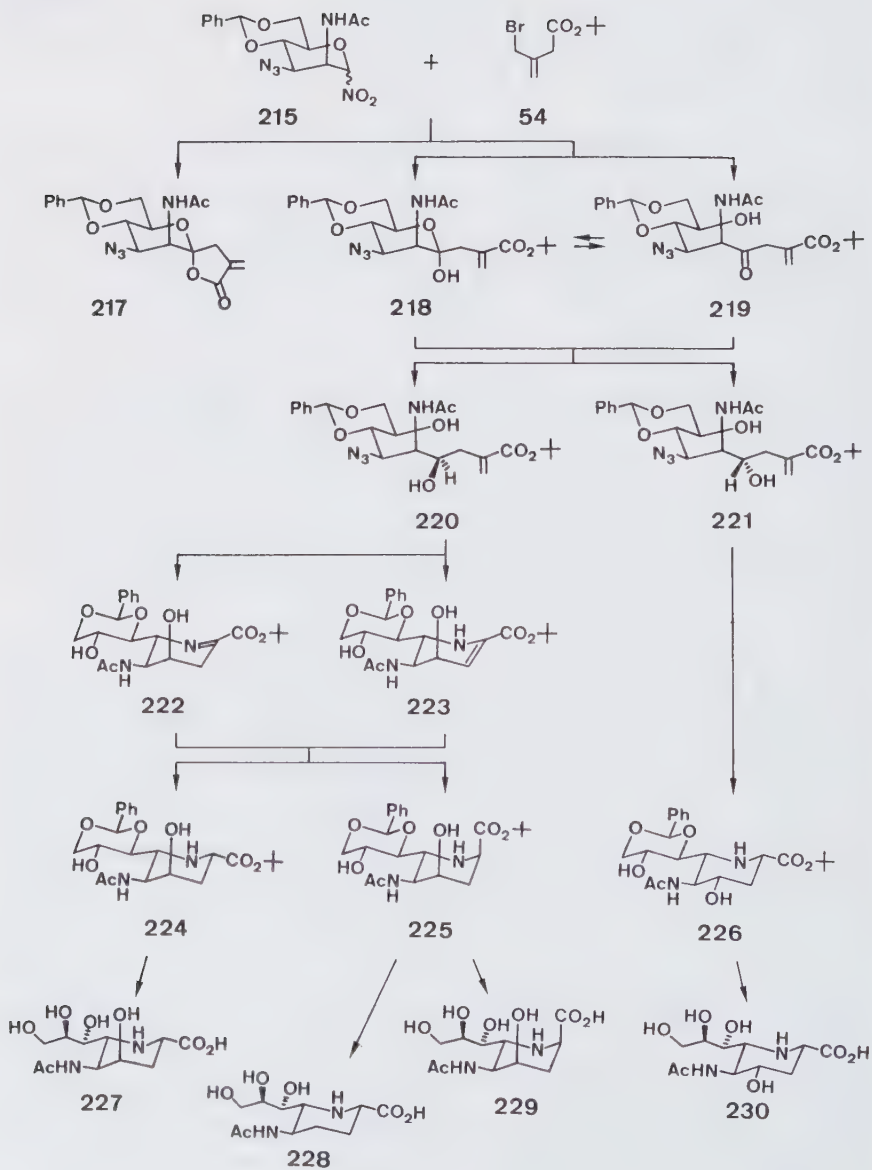
The manno-configuration of the anomeric 2-acetamido-3-azido-sugars 215 was assigned from the ^1H -NMR coupling constants. The values $J_{1,2} = 1.2$ and 2.7 Hz, $J_{2,3} = 5.0$ and 5.2 Hz, and $J_{3,4} = 10.0$ Hz for the α and β anomers, respectively, prove the configuration. IR-absorptions at 2110 , 1688 and 1560 cm^{-1} confirm the presence of the azido-, acetamido- and nitro groups.

The olefin 216 does not show an azide band in its IR-spectrum. It is further characterized in the ^{13}C -NMR spectrum by a doublet at ca. 100 ppm for C(3) and a singlet for C(4) at 153.5 ppm. These values agree well with the proposed enol ether structure of 216. (In the IR-spectrum, the expected absorption band of the enol ether function is obscured by a strong amide band). In spite of the trans diaxial arrangement of H-C(4) and the trifluorosulfonyl group, the olefin 216 is only formed as a by-product.

The N-acetylmannosamine derivative 215 reacted at 0° in THF and in the presence of DBU with tert-butyl 2-(bromomethyl)prop-2-enoate 54 (Michael addition followed by β -elimination) to an intermediate (89%), which was hydrolyzed in a mixture of CH_3CN and citrate buffer (pH 5.5) at r.t. to the crystalline tert-butyl 4-nonulosonate 218 and the γ -lactone 217 (Scheme 31). As shown by ^1H -NMR-spectroscopy, a sample of crystalline 218 freshly dissolved in (D_6) acetone does not equilibrate with 219 within 45 min, while in (D_6) DMSO a 6:4 mixture of 218/219 was already detectable after 5 min. The pyranose 218 shows in the ^{13}C -NMR spectrum a singlet at 99.17 ppm for C(4) (anomeric centre), while the open-chain tautomer 219 shows a C(4) signal at 204.03 ppm as a singlet.

The ^1H - and ^{13}C -NMR spectra of the γ -lactone 217 show the absence of a tert-butyl group and a singlet for C(4) at 105 ppm. An IR band at 1786 [267] confirms the presence of a α -methylene γ -lactone. In solution, the lactone 217 decomposes within about 14 days at r.t.; in solid state it is more stable. It could, however, only be crystallized after an extensive chromatographic purification.

Scheme 31



Reduction of the tert-butyl 4-nonulosonate 218/219 with NaBH₄/HOAc in THF/H₂O 4:1³⁵⁾ gave a 84:16 mixture of the diols 220 and 221³⁶⁾. The configuration of the diols 220 and 221, respectively, was deduced by transformation of 220 into the 6-amino-analogues of 4-epi-Neu5Ac 224 and 225, respectively, and of the diol 221 into the 6-amino-analogue of Neu5Ac 226.

Although a disadvantage from the preparative viewpoint, this result contributes to an understanding of the influence of the reaction conditions upon the diastereoselectivity of the NaBH₄-reduction of the related hemiacetals 143 and 145, respectively (Chap. 5).

It is not sufficient to disturb the presumed hydrogen bond between the NHAc group and the C(4) carbonyl group (leading to the undesired epimer) or to form a new reagent to obtain the desired stereoisomer. (Evidence for such a H-bond is found in the 'Diplomarbeit' of M. Christen [171], who showed that the reduction conditions leading to a 7:3 ratio from 143 (see Chap. 5.2) give a 1:2 ratio from the N-methyl-analogue of 143). Apparently, the C(6)-OH group participates in the reduction step, either by forming a H-bond with the C(4) carbonyl group or by reaction of the C(6) OH group with a newly formed reductive agent and intramolecular delivery of a hydride.

Ozonolysis of the diol 220 and subsequent reduction of the azido function by transfer hydrogenation (Pd/C, HCOONH₄ in MeOH) gave a 55:45 mixture of the imine 222 and the enamine 223 (94% from 220) [268]. Reduction of the azido group [269] (in the ozonolysis product) with propan-1,3-dithiol [270], EtOH/aq. TiCl₃ [271] or RaNi [272] resulted in each case in complex mixtures. Conversion of the azido group into an amino group by the Staudinger reaction [273-275] occurred only upon heating the mixture up to 60° for 3 d and gave also a mixture of the imine 222 and the enamine 223 (68% from the diol 220).

35) Dioxane/H₂O 4:1 (see Chap. 5.2) gave the same result.

36) The ratio of the epimeric mixture of 220/221 was always 80:20 or worse and independent of the solvent (protic, aprotic, with water or without water).

In the ^{13}C -NMR spectrum, the imine 222 is characterized by a singlet at 161.80 ppm for C(2) and a triplet at 36.34 ppm for C(3); and the enamine 223 by a singlet at 136.64 ppm for C(2) and a doublet for C(3) at 103.96 ppm. An absorption at 1710 cm^{-1} in the IR-spectrum of 222/223 indicates the presence of an α,β -unsaturated ester.

Hydrogenation of a mixture of 222/223 (AcOEt/benzene, 1d) in the presence of Pd/C yielded the D-erythro-L-allo-configured piperidine derivative 224 (75%) and the D-erythro-L-altro-configured piperidine derivative 225 (19%). The configuration of 224 and 225, respectively, was deduced from the ^1H -NMR-spectra. (224: $J_{2,3} = 3.0$ and 12.0 Hz, and $J_{4,5} = 2.8$ Hz; 225: $J_{2,3} = 3.0$ and 6.0 Hz, and $J_{4,5} = 2.8$ Hz). All other coupling constants were very similar to those obtained for the corresponding 4-epi-Neu5Ac-derivative 154 (see Chap. 5.2 and experimental part).

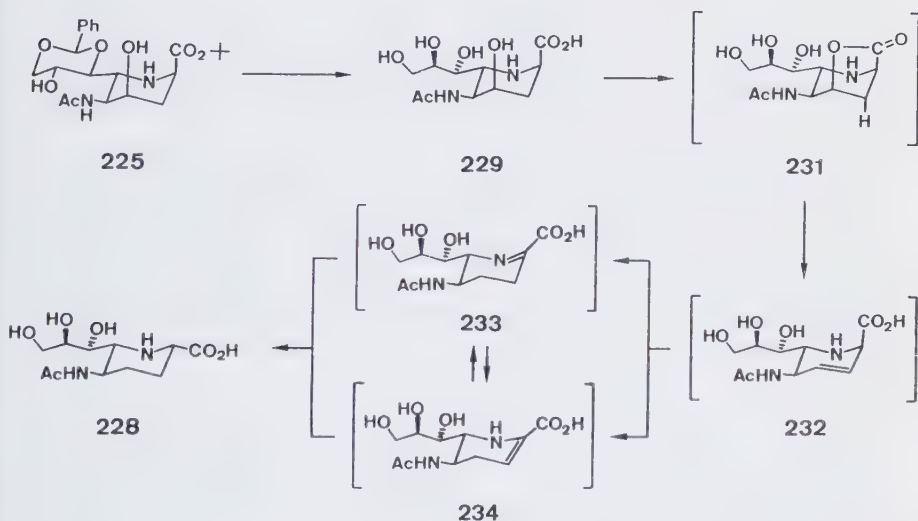
Deprotection of 224 with CF_3COOH followed by ion-exchange-chromatography gave the amino acid 227 in a 72% yield as a colourless microcrystalline solid. Under similar conditions, the piperidine derivative 225 did not give the amino acid 229 but the C(4) deoxygenated amino acid 228 (43%). The formation of the deoxygenated product 228 can be explained by assuming a conversion of the amino acid 229 into a γ -lactone 231 (Scheme 32). Elimination might then give a γ,δ -unsaturated acid 232. This acid may isomerize giving 234, which would equilibrate with the corresponding imine 233. Finally, reduction of 233 with formic acid on Dowex 1X8 would give 228. The modest yield is presumably due to incomplete elution by HCOOH from the ion exchanger column³⁷⁾.

The amino acid 228 is characterized in the ^{13}C -NMR-spectrum by two signals at 31.87 and 27.71 ppm for the C(3) and C(4) methylene groups, respectively. The ^1H -NMR-spectrum of 228 is very similar to the one of

³⁷⁾ In the case of 227 and 229, elution with aq. HCOOH was less effective than with aq. HCl .

4-deoxy-Neu5Ac, particularly the chemical shifts of $H_{ax}-C(3)$, $H_{ax}-C(4)$, $H_{eq}-C(4)$ and $H_{eq}-C(3)$ are almost identical (see Chap. 5.2 and experimental part). We found $J_{4,5} = 11.0$ and 4.2 Hz confirming the expected configuration at C(5) and $J_{2,3} = 13.0$ and 3.2 Hz establishing the equatorial orientation of the carboxyl group.

Scheme 32



The D-erythro-L-altro-configured amino acid 229 was obtained from 225 by saponification of the tert-butyl ester with NaOH, followed by acidic debenzylidenation and a final purification by ion-exchange-chromatography on Dowex 1X8. The configuration of the amino acid 229 is secured by the $J_{2,3}$ -coupling constants of 1.5 and 7.0 Hz, respectively, in its 1H -NMR-spectrum.

Ozonolysis of the D-glycero-D-galacto-configured diol 221, and subsequent reduction of the azido group (H_2 , Pd/C) gave the benzylidene protected amino acid ester 226 in 58% yield. An imine/enamine intermediate corresponding to 222/223 was never observed. Reduction of the azido group by transfer hydrogenation (see above) did not lead to an imine/enamine either, but gave a complex mixture, which was simplified by subsequent hydrogenation (H_2 , Pd/C) to give the amino acid ester 226. The C(2)-epimer of 226 could not be isolated, although three other minor products were observed on TLC next to 226. The D-erythro-L-gluco-configuration of 226 was proven by the values of the vicinal coupling constants namely $J_{2,3eq} = 2.8$ Hz, $J_{2,3ax} = 11.8$ Hz and $J_{4,5} = 10.0$ Hz. All other coupling constants were very similar to those of the corresponding Neu5Ac-derivative (see Chap. 5.2).

Similarly to 225, the piperidine 226 was deprotected by saponification of the tert-butyl ester (NaOH), followed by acidic removal of the benzylidene group and an ion exchange chromatography to give the amino acid 230 (98%). Apart from $J_{2,3}$ (13.0 and 3.0 Hz), the 3J -coupling constants were very similar to those of Neu5Ac.

EXPERIMENTAL PART

1. General remarks

Usual workup implies washing of the organic layer with brine, drying (MgSO_4), filtration and concentration in a rotary evaporator under reduced pressure below 40° . The drying of the residue was effected under reduced pressure (10^{-2} - 10^{-5} mbar) for several hours at r.t.

Thin layer chromatography (TLC) was effected on 0.25 mm precoated silica gel plates (Kieselgel 60 F₂₅₄, Merck). Substances were detected by spraying the plates with an acidic iodine solution³⁸⁾ or with a 10% solution of phosphomolybdic acid in EtOH, in each case followed by heating at about 200° .

Column chromatography was performed on silica gel (Kieselgel 60, Merck), 70-230 mesh, for normal chromatography, 230-400 mesh, for flash-chromatography (FC) [276] or MPLC according to Loibner and Seidl [190].

Preconditioned column means: the indicated solvent was passed through the column until it appeared visually homogeneous.

GLC was performed on a Hewlett-Packard 5880 apparatus using a SP 2100 column.

³⁸⁾ Mixture 1:1 (v/v) of a solution containing 10 g of iodine and 100 g of KI in water (1 liter) with a 20% solution of H_2SO_4 .

Analytical HPLC was performed on a Kontron apparatus (LC pump 410) with a UV detector.

Preparative HPLC was performed on a Du Pont 8800 apparatus; detection by UV-absorption or differential refractometer.

Melting points (M.p.) were determined on a Büchi 510 apparatus and are uncorrected.

Optical rotations ($[\alpha]_D$) were measured on a Perkin-Elmer 241 polarimeter at 25° in a 1 dm cell at 365, 436, 564, 578 and 589 nm; the specific rotation at 589 nm was determined using a regression curve, unless an ORD effect was noted in which case the value obtained at 589 nm was considered.

Infrared Spectra (IR) were recorded on a Perkin-Elmer 298 spectrometer. Unless stated otherwise, IR spectra refer to 3% CHCl₃ solutions. The wave number in cm⁻¹ were characterized by s (strong), m (medium), w (weak), sh (shoulder), and br. (broad).

Nuclear Magnetic Resonance Spectra (NMR) were recorded on a Varian-FT-80A (¹H (80 MHz)), Varian-HA-100 (¹³C (25.2 MHz)), Varian-XL-200 (¹H (200 MHz), ¹³C (50 MHz)), or Bruker-AM-400 spectrometer (¹H (400 MHz), ¹³C (100 MHz)); CDCl₃ solutions unless otherwise specified.

The chemical shifts are reported in ppm relative to TMS as internal standard or external reference when D₂O was the solvent. Multiplicities (s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet)), coupling constants J in Hz, relative intensities and assignments are given in parenthesis.

Mass Spectra (MS) were registered on a Varian-112S (EI: 70 eV); CI: isobutane) or a Varian-711 spectrometer (FAB, bombardement with 8-keV Xe-atoms, glycerol matrix).

Quality of the solvents and reagents.

The common solvents were distilled before use. Tetrahydrofurane (na), methylene chloride (P_2O_5), methanol (Mg), and ethanoi (Mg) were distilled in an apparatus with continous distillation.

Acetonitrile was kept over $CaCl_2$ (24 h), distilled from P_2O_5 , and stored over molecular sieve 3 Å.

Amberlite IRA-93 was activated by washing it sequentially with 0.5 N NaOH, H_2O (bidest), MeOH, ether and drying it over P_2O_5 at 10^{-2} mbar.

α '-Azaisobutyronitrile (AIBN) was obtained from Fluka and used without further purification.

Benzene was extracted with conc. H_2SO_4 , washed with H_2O and distilled from Na.

tert-Butyl 2-(bromomethyl)prop-2-enoate (**54**) was distilled before use [185].

Chloroacetic anhydride ($ClCH_2CO)_2O$) was obtained from Fluka and used without further purification.

Chloroform (for IR and optical rotation) was extracted with conc. H_2SO_4 , left 18 h over $CaCl_2$ and then distilled from $CaCl_2$.

DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) was obtained from Fluka.

Dimethylformamid (DMF) was distilled from CaH_2 .

Dioxane was distilled from Na.

Dowex 1X8 ($HCOO^-$) and

Dowex 50X4 (H^+) were washed and activated according to [277].

Ethyl acetate was distilled from K_2CO_3 and then from P_2O_5 .

Formic acid ($HCOOH$) was distilled from phthalic anhydride.

2-Methoxypropene was obtained from Fluka.

Ozone was produced from a Fischer generator.

Palladium over charcoal (Pd/C) was obtained from Fluka (10%).

Pyridine was distilled from CaH_2 .

Tributyltin hydride (Bu_3SnH) was prepared according to the procedure of Kuivila [278].

2. Stereoelectronic Control in the Reductive Denitration of Tertiary Nitro Ethers. A Synthesis of 'C-Glycosides'

Acetylations were carried out at 0° in pyridine/Ac₂O 2:1. Excess of pyridine and Ac₂O were removed at 40°/0.5 Torr.

General Procedure for the Reductive Denitration. A 3-necked flask equipped with a condenser, a dropping funnel and a gas inlet tube was dried at 120° and then cooled under Ar. Freshly distilled Bu₃SnH (5 mmol) and AIBN (0.2-0.4 mmol) in benzene (5 ml) were added dropwise within 1-2 h to a degassed, boiling solution of the nitro compound (1 mmol) in benzene (15 ml). When TLC indicated the disappearance of the nitro compound, the cooled mixture was concentrated and the residue was purified by chromatography on SiO₂.

1-O-Acetyl-3,4,5,7-tetra-O-benzyl-2-deoxy-2-nitro-D-gluco-heptulopyranoses (64 and 65). To a stirred solution of 1.5 g (2.6 mmol) of 2,3,4,6-tetra-O-benzyl-1-deoxy-1-nitro-D-glucopyranose (**63**) [121] in 50 ml anh. MeOH were added 790 mg (26 mmol) of paraformaldehyde and 72 mg (0.5 mmol) of K₂CO₃. The mixture was stirred for 3 h at r.t., poured into 150 ml Et₂O and extracted with H₂O. The crude product was purified by flash chromatography (60 g SiO₂, Et₂O/hexane 1:2) yielding 870 mg (56%) of a material, 500 mg (0.83 mmol) of which were acetylated affording after FC (25 g SiO₂, Et₂O/hexane), 530 mg (quant.) of **64/65**. R_f 0.45 (hexane/AcOEt 2:1). The anomers were separated by prep. HPLC (Zorbax-Sil, Et₂O/hexane 4:1, k'=2.1 for **64**, k'=2.5 for **65**).

Data of 64. $[\alpha]_D^{25} = +55^\circ$ (c = 1.6, CHCl₃). IR: 3085w, 3065w, 3030sh, 3005m, 2910w, 2870m, 1751s, 1567s, 1552s, 1495m, 1452s, 1382sh, 1365s, 1220s, 1120sh, 1091s, 1072s, 1028s, 693m. ¹H-NMR (90 MHz): 7.45-7.10 (m, 20 arom. H); 4.85-4.35 (m, 11 H); 4.30-3.70 (m, 5 H); 1.93 (s, CH₃). ¹³C-NMR (25.2 MHz): 169.45(s); 138.04(s); 137.67(s); 137.41(s); 136.54(s); 128.43(d); 128.33(d); 128.21(d); 128.05(d); 127.93(d); 127.82(d); 127.74(d); 127.63(d); 127.47(d); 110.62(s); 79.51(d); 76.54(d); 76.12(d); 75.90(d); 75.23(t); 74.32 (t); 73.95(t); 73.23(t); 68.09(t); 63.83(t); 20.56(q). EI-MS: 552 (≤0.2), 503 (≤0.1), 444 (≤0.1), 429 (≤0.1),

413 (≤ 0.1), 397 (≤ 0.1), 353 (≤ 0.1), 307 (≤ 0.2), 271 (≤ 0.3), 259 (≤ 0.5), 253 (2), 181 (6), 91 (100), 43 (5). Anal. calc. for $C_{37}H_{39}NO_9$ (641.73): C 69.25, H 6.13, N 2.19; found: C 69.13, H 6.40, N 2.19.

Data of 65. $[\alpha]_D^{25} = +2.4^\circ$ ($c = 1.6$, $CHCl_3$). IR: 3090w, 3065w, 3030sh, 3005m, 2955m, 2925m, 2870m, 1748s, 1566s, 1558sh, 1495w, 1452s, 1381m, 1365s, 1280sh, 1220s, 1158m, 1073s, 1028s, 693m. 1H -NMR (90 MHz): 7.50-7.05 (m, 20 arom. H); 5.10-4.40 (m, 10 H); 4.35-3.60 (m, 6H); 1.98 (s, CH_3). ^{13}C -NMR (25.2 MHz): 169.83(s); 138.02(s); 137.59(s); 137.34(s); 136.45(s); 128.47(d); 128.38(d); 128.30(d); 128.23(d); 128.13(d); 127.83(d); 127.76(d); 127.70(d); 127.48(d); 109.84(s); 81.18(d); 79.17(d); 76.72(d); 76.17(d); 74.80(t); 73.98(t); 73.80(t); 73.40(t); 68.68(t); 62.24(t); 20.59(q). MS: indistinguishable from that of 64. Anal. calc. for $C_{37}H_{39}NO_9$ (641.73): C 69.25, H 6.13, N 2.19; found: C 69.45, H 6.27, N 2.04.

1-O-Acetyl-2,6-anhydro-3,4,5,7-tetra-O-benzyl-D-glycero-D-gulo-heptitol (66). A) From 64. Denitration of 285 mg (0.44 mmol) of 64 gave, after chromatography (30g SiO_2 , $AcOEt$ /hexane 1:4) 250 mg (95%) of 66. $[\alpha]_D^{25} = -4.4^\circ$ ($c = 1.3$, $CHCl_3$). IR: 3090w, 3065w, 3030sh, 3005m, 2910sh, 2870m, 1740s, 1497m, 1455m, 1385sh, 1365m, 1310w, 1220s, 1150m, 1095s, 1065s, 1030s, 695m. 1H -NMR (90 MHz): 7.45-7.07 (m, 20 arom. H); 5.00-4.05 (m, 10 H); 3.90-3.30 (m, 7 H); 2.00 (s, CH_3). ^{13}C -NMR (25.2 MHz): 170.53(s); 138.35(s); 138.10(s); 137.92(s); 137.62(s); 128.36(d); 128.31(d); 128.27(d); 128.20(d); 127.93(d); 127.77(d); 127.62(d); 127.51(d); 127.44(d); 87.10(d); 79.14(d); 78.28(t); 78.03(d); 77.00(t); 75.50(d); 75.01(d); 74.97(t); 73.40(t); 68.85 (t); 63.47(t); 20.91(q). EI-MS: 505 (2), 399(1), 291(1), 181(8), 91(100), 43(5). Anal. calc. for $C_{37}H_{40}O_7$ (596.76): C 74.47, H 6.76; found: C 74.41, H 6.64.

B) From 65. Denitration of 55 mg (0.086 mmol) of 65 gave 45 mg (87%) of 66. IR and 1H -NMR spectra indistinguishable from those in A.

2,6-Anhydro-1,3,4,5,7-penta-O-benzyl-D-glycero-D-gulo-heptitol (68). A solution of 130 mg (0.22 mmol) of 66 was kept at r.t. in 7.5 ml of 2% NaOMe in MeOH for 30 min (TLC, AcOEt/hexane 1:2). The solvent was evaporated and the residue was purified by flash chromatography (15g SiO₂, Et₂O) giving 67 (quant.) as a colourless oil, which was crystallized from AcOEt/hexane. Colourless needles, m.p. 93.0-93.5°, $[\alpha]_D^{25} = +8.6^\circ$ (c = 1.1, CHCl₃). IR: 3595w, 3460 br., 3090w, 3070w, 3035sh, 3005m, 2920sh, 2870m, 1496m, 1454s, 1398w, 1362m, 1306w, 1220 br., 1146s, 1098s, 1064sh, 1028s, 1000m, 694m. ¹H-NMR (90 MHz): 7.45-7.05 (m, 20 arom. H); 4.97-4.43 (m, 4 CH₂-Ph); 4.00-3.20 (m, 9 H); 2.15 (br., OH).

A mixture of 120 mg (0.22 mmol) of 67, 120 mg (2.2 mmol) of KOH, 250 µl (2.2 mmol) of benzylchloride and 3 ml dioxane was kept at 50° for 15 min and then diluted with 5 ml of Et₂O. The KOH was filtered off and the filtrate was concentrated. Flash chromatography (25g SiO₂, hexane/Et₂O 3:1) afforded 123 mg (86%) of 68 as colourless crystals, m.p. 55.0-55.5°. IR: 3090w, 3065m, 3030sh, 3005m, 2910sh, 2870s, 1496m, 1454s, 1362m, 1310w, 1154m, 1092s, 1060s, 1030s, 694m. ¹H-NMR (90 MHz): 7.45-7.05 (m, 25 arom. H); 4.90-4.45 (m, 5 CH₂-Ph); 3.87-3.30 (m, 9 H). ¹³C-NMR (25.2 MHz): 138.45(s); 138.07(s); 137.96(s); 128.14(d); 128.10(d); 127.69(d); 127.56(d); 127.43(d); 127.31(d); 87.07(d); 79.00(d); 78.26(d); 75.36(t); 74.82(t); 73.31(t); 69.04(t). EI-MS: 553 (3), 339 (2), 249 (2), 181 (17), 91 (100). Anal. calc. for C₄₂H₄₄O₆ (644.82): C 78.23, H 6.88; found: C 77.96, H 6.67.

1-O-Acetyl-3,4,5,7-tetra-O-benzyl-2-deoxy-2-nitro-D-manno-heptulopyraoses (70 and 71). A solution of 285 mg (0.5 mmol) of 2,3,4,6-tetra-O-benzyl-1-deoxy-1-nitro-D-manno-pyranose (69) [121] in 5 ml of DMF, 150 mg (5 mmol) of paraformaldehyde and 28 mg (0.2 mmol) K₂CO₃ was kept at r.t. until disappearance of 69 (20 min) as indicated by TLC (hexane/AcOEt 2:1) and then poured into 25 ml of Et₂O. Normal workup gave a residue, which was dried i.v. and acetylated (0°, 30 min). TLC (hexane/Et₂O 3:1) showed 4 spots, the major ones (=fastest and slowest moving ones) corresponded to 70 and 71. The residue obtained after evaporation of the solvent was separated by FC (40 g SiO₂, Et₂O/hexane 1:4), giving 205 mg (65%) of 70 (α-D), 42 mg (12%) of 71 (β-D) and 56 mg of two nonidentified by-products. All compounds were slightly yellow oils.

Data of 70. $[\alpha]_D^{25} = +54.5^\circ$ ($c = 1.4$, CHCl_3). IR: 3090w, 3060w, 3030sh, 3005m, 2925m, 2870m, 1752s, 1560sh, 1554s, 1495s, 1454m, 1365m, 1220s, 1104s, 1072s, 1028m, 694m. $^1\text{H-NMR}$ (90 MHz): 7.43-7.03 (m, 20 arom. H); 5.10-4.02 (m, 12 H); 3.95-3.70 (m, 3 H); 3.60 (dd, $J=9.0$ and 2.5, H-C(4)); 1.97 (s, CH_3). $^{13}\text{C-NMR}$ (25.2 MHz): 169.17(s); 138.07(s); 137.77(s); 137.45(s); 137.20(s); 128.41(d); 128.36(d); 128.20(d); 128.17(d); 127.93(d); 127.84(d); 127.77(d); 127.70(d); 127.60(d); 127.39(d); 111.95(s); 79.91(d); 77.88(d); 75.54(t); 74.95(t); 74.43(d); 73.34(t); 73.06(t); 72.83(d); 68.28(t); 64.51(t); 20.49(q). EI-MS: 505 (0.3), 503 (0.5), 413 (0.5), 373 (2); 304 (3), 253 (3), 181 (21), 91 (100), 43 (20). Anal. calc. for $\text{C}_{37}\text{H}_{39}\text{NO}_9$ (641.73): C 69.25, H 6.13, N 2.19; found: C 68.99, H 5.94, N 2.13.

Data of 71. $[\alpha]_D^{25} = -38.8^\circ$ ($c = 1.7$, CHCl_3). IR: 3085w, 3060w, 3030sh, 3005m, 2955m, 2925m, 2870m, 1754s, 1577s, 1558sh, 1453m, 1376m, 1215s, 1097s, 1052s, 1028s. $^1\text{H-NMR}$ (90 MHz): 7.45-7.05 (m, 20 arom. H); 4.95-3.60 (m, 16 H); 2.00 (s, CH_3). $^{13}\text{C-NMR}$ (25.2 MHz): 169.53(s); 138.30(s); 137.79(s); 137.36(s); 137.11(s); 128.50(d); 128.24(d); 128.15(d); 128.10(d); 128.01(d); 127.83(d); 127.78(d); 127.65(d); 127.53(d); 127.44(d); 127.29(d); 126.99(d); 109.46(s); 80.83(d); 77.89(d); 76.25(d); 75.91(t); 74.80(t); 74.11(d); 73.56(t); 73.20(t); 68.56(t); 60.97(t); 20.39(q). EI-MS: 594 (≤ 1), 552 (≤ 1), 443 (≤ 1), the other peaks corresponded to those of 70. Anal. calc. for $\text{C}_{37}\text{H}_{39}\text{NO}_9$ (641.73): C 69.25, H 6.13, N 2.19; found: C 69.54, H 6.36, N 2.16.

1-O-Acetyl-2,6-anhydro-3,4,5,7-tetra-O-benzyl-D-glycero-D-galacto-heptitol (72). A) From 70. Denitration of 51 mg (0.08 mmol) of 70 was terminated after 2.5 h (TLC, hexane/AcOEt 3:1). Evaporation of the solvent and flash chromatography of the residue (15g SiO_2 , hexane/Et₂O 2:1) gave 40 mg (84%) of 72, $[\alpha]_D^{25} = +5.7^\circ$ ($c = 1.3$, CHCl_3). IR: 3090w, 3065w, 3030sh, 3005m, 2870m, 1740s, 1495w, 1453s, 1377s, 1225s, 1135sh, 1110s, 1050s, 1028s, 694m. $^1\text{H-NMR}$ (90 MHz): 7.50-7.05 (m, 20 arom. H); 5.15-4.40 (m, 8 H); 4.40-3.32 (m, 9 H); 1.97 (s, CH_3). $^{13}\text{C-NMR}$ (25.5 MHz): 170.44(s); 138.31(s); 138.14(s); 128.36(d); 128.19(d); 128.07(d); 127.95(d); 127.78(d); 127.56(d); 127.44(d); 84.83(d); 79.88(d); 75.74(d); 75.22(d,t); 74.18(t); 73.42(d,t); 72.63(t); 69.45(t); 63.59(t); 20.91(q). EI-MS: 505

(2), 446 (≤ 0.2), 413 (≤ 0.2), 291 (≤ 0.5), 201 (1), 181 (7), 105(3), 91 (100), 57 (22), 43 (15). Anal. calc. for $C_{37}H_{40}O_7$ (596.73): C 74.47, H 6.76; found: C 74.63, H 7.01.

B) From 71. In an analogous way, denitration of 60 mg (0.09 mmol) of 71 gave after flash chromatography (25 g SiO_2 , hexane/ Et_2O 2:1) 27 mg (49%) 72. The 1H -NMR and IR spectra of this product were identical with those in A.

2,6-Anhydro-D-glycero-D-galacto-heptitol (74) [150]. A solution of 480 mg (0.8 mmol) of 72 in 40 ml of a 2% solution of NaOMe in MeOH was kept for 1 h at r.t. Usual workup (Et_2O , H_2O) gave 428 mg (96%) of 73. Slightly yellow oil, $[\alpha]_D^{25} = +15.1^\circ$ ($c = 1.5$, $CHCl_3$). IR: 3590w, 3480 br., 3085w, 3065w, 3030sh, 3002m, 2865m, 1495m, 1454m, 1362m, 1086s, 1047s, 1028s, 909s, 690m. 1H -NMR (90 MHz): 7.48-7.05 (m, 20 arom. H); 5.07-4.40 (m, 4 CH_2 -Ph); 4.07-3.25 (m, 9 H); 1.95 (br., OH).

A solution of 120 mg (0.22 mmol) of 73 in 2.2 ml of AcOH was hydrogenated for 2.5 h in the presence of 60 mg of 10% Pd/C. After disappearance of the starting material (TLC, $EtOH/H_2O$ 7:3), the precipitate was filtered off (Celite) and washed with $EtOH$. The filtrate was concentrated and the residue was purified by chromatography (10 g SiO_2 , $EtOH/H_2O$ 9:1) and then by distribution between H_2O and $AcOEt$. Lyophilisation of the aq. solution yielded 36 mg (85%) of 74 as a colourless solid, which was crystallized from $EtOH$, m.p. 129-130° ([150]: 142-144°), $[\alpha]_D^{25} = -33.4^\circ$ ($c = 1.5$, H_2O) ([150]: -33.6°). Anal. cal. for $C_7H_{14}O_6$ (194.19): C 43.30, H 7.27; found: C 43.55, H 7.35.

trans- and cis-4-(tert-Butyl)-1-nitrocyclohexylmethyl acetates (76 and 77, respectively). A stirred solution of 110 mg (0.59 mmol) of 1-(tert-butyl)-4-nitrocyclohexane (75) [152], 178 mg (5.9 mmol) of paraformaldehyde and 16 mg (0.12 mmol) of K_2CO_3 in 3.5 ml of anh. MeOH was kept at r.t. until 75 had disappeared (3/4 h; TLC, hexane/ $AcOEt$ 7:3). The mixture was diluted with 10 ml of Et_2O . Usual workup gave a colourless solid (80 and 81). Acetylation of this solid gave, after flash chromatography (20 g SiO_2 , hexane/ $AcOEt$ 20:1), 104 mg (68%) of 76 and 46 mg (30%) of 77.

Data of 76. M.p. 84-86° (from hexane). IR: 3020w, 2960s, 2910sh, 2870m, 2845w, 1746s, 1736sh, 1545s, 1476w, 1458m, 1450m, 1428m, 1375m, 1365s, 1350w, 1336w, 1324w, 1220s, 1152s, 1142m, 838w. ¹H-NMR (90 MHz): 4.29 (s, CH₂OAc); 2.72 (d, J=12, H_{eq}-C(2), H_{eq}-C(6)); 2.11 (s, CH₃); 1.95-1.00 (m, 7 H); 0.90 (s, t-bu). ¹³C-NMR (25.5 MHz): 169.78(s); 89.11(s); 69.75(t); 46.82(d); 32.31(s); 31.58(t); 27.30(q); 22.55(t); 20.46(q). EI-MS: 211 (≤1), 201 (4), 154 (6), 135 (6), 111 (7), 95 (21), 94 (25), 93 (41), 81 (10), 79 (16), 57 (100). Anal. calc. for C₁₃H₂₃NO₄ (257.33): C 60.68, H 9.01, N 5.44; found: C 60.91, H 9.06, N 5.29.

Data of 77. Colourless oil, b.p. 125°/0.01 Torr. IR: 3020w, 2965s, 2910sh, 2875m, 1748s, 1736sh, 1545s, 1480sh, 1462m, 1448m, 1388m, 1374s, 1368s, 1339w, 1272sh, 1220s, 1050s. ¹H-NMR (90 MHz): 4.53 (s, CH₂OAc); 2.47-1.50 (m, 6 H); 2.03 (s, CH₃); 1.43-0.97 (m, 3 H); 0.87 (s, t-bu). ¹³C-NMR (25.2 MHz): 169.71(s); 89.06(s); 63.23(t); 46.67(d); 32.07(s); 31.63(t); 27.29(q); 23.67(t); 20.36(q). MS: indistinguishable from that of 76. Anal. calc. for C₁₃H₂₃NO₄ (257.33): C 60.68, H 9.01, N 5.44; found: C 60.68, H 8.79, N 5.19.

The corresponding alcohols 80 and 81 were obtained by flash chromatography (SiO₂, hexane/AcOEt 10:1) of the crude addition product.

trans-4-(tert-Butyl)-1-nitrocyclohexylmethanol (80). M.p. 125-126°. IR: 3620m, 2965s, 2875m, 1538s, 1462m, 1447m, 1433m, 1370m, 1100w, 1060m, 840w. ¹H-NMR (90 MHz): 3.75 (s, CH₂O); 2.68 (d, J=12, H_{eq}-C(2), H_{eq}-C(6)); 2.20 (br., OH); 1.90-0.93 (m, 7 H); 0.87 (s, t-bu).

cis-4-(tert-Butyl)-1-nitrocyclohexylmethanol (81). M.p. 97.0-97.5°. IR: 3600m, 2970s, 2880m, 1538s, 1465m, 1400w, 1372m, 1344w, 1066s, 983w, 930w, 867w. ¹H-NMR (90 MHz): 4.00 (d, J=6.0, CH₂O); 2.53-0.98 (m, 10 H); 0.88 (s, t-bu).

trans- and cis-4-(tert-Butyl)-cyclohexylmethyl acetates (78 and 79, respectively). A) From 76. Acetate 76 (100 mg, 0.39 mmol) was treated with Bu₃SnH as described above. After 7 h, TLC (hexane/AcOEt 10:1) indicated the disappearance of 76. The mixture was concentrated and the residue was

purified by flash chromatography (25 g SiO₂, hexane/AcOEt 40:1) yielding 78 mg (95%) of 78/79 (83:17, by ¹H-NMR and GLC (140°)), colourless oil, b.p. (mixture) 125-130°/15 Torr. IR: 2940s, 2920sh, 2880m, 1727s, 1468w, 1450w, 1392w, 1365m, 1248s, 1220s, 1034m, 1025sh. ¹H-NMR (90 MHz): 4.05 (d, J=7.5, 0.17 CH₂OAc); 3.84 (d, J=6.0, 0.83 CH₂OAc); 2.03 (s, CH₃); 1.96-0.90 (m, 10 H); 0.86 (s, t-bu); ([153]: 4.03 (CH₂OAc of 79) and 3.81 (CH₂OAc of 78)). ¹³C-NMR (25.2 MHz): 78: 170.93(s); 69.64(t); 48.03(d); 37.24(d); 32.41(s); 30.12(t); 27.55(q); 26.66(t); 20.91(q). 79: 164.05, 65.42, 48.30, 32.51, 31.69, 27.71, 27.47, 22.04, 20.99, ([153]: 64.81 (C(1) of 79); 69.54 (C(1) of 78)). EI-MS: 157 (2), 156 (2), 152 (9), 137 (8), 97 (42), 96 (32), 95 (40), 94 (11), 81 (56), 79 (15), 68 (12), 67 (34), 61 (14), 57 (100), 56 (46), 54 (11), 43(73). Anal. calc. for C₁₃H₂₄O₂ (212.34): C 73.54, H 11.39; found: C 73.26, H 11.67.

B) From 77. An analogous denitration of 190 mg (0.74 mmol) of 77 gave 143 mg (91%) of 78/79 with the same ratio of the trans- to cis-isomer.

The mixture 78/79 was deacetylated by 2% NaOMe in MeOH giving 82/83. A mixture of 82 and 83 (81:19 by ¹H-NMR) was also obtained by denitration of 80 or 81. B.p. (mixture) 120°/15 Torr. IR (CCl₄): 3640m, 3350 br., 2940s, 2860s, 1478m, 1468m, 1449m, 1392m, 1364s, 1236w, 1082w, 1032s, 1021m, 1008m. ¹H-NMR (90 MHz, CCl₄): 3.48 (d, J=7.5, 0.19 CH₂O); 3.30 (d, J=6.0, 0.81 CH₂O); 2.89 (s, OH); 2.00-0.55 (m, 10 H); 0.86 (s, t-bu); ([154]: 3.47 (d, J=7, CH₂O of 83); 3.29 (d, J=5, CH₂O of 82)).

The mixture 82/83 was transformed into the toluenesulfonates 84/85 [155], which were separated by analytical HPLC (Zorbax-NH₂, hexane/THF 97:3) into 84, m.p. 95-96° ([155]: 95.5-96.0°) and 85, m.p. 82-83° ([155]: 83-84°).

4,7-Anhydro-2,3-dideoxy-5,6:8,9-di-O-isopropylidene-D-glycero-D-galactonononitrile (87). To the degassed solution of 200 mg (0.58 mmol) of 86 [122] and 20 mg (0.12 mmol) of AIBN in 7.6 ml of benzene was added slowly (1.5 h) a solution of 770 µl (2.90 mmol) of Bu₃SnH in 2.4 ml of benzene. The mixture was kept under reflux until 86 had disappeared (6 h,

TLC, hexane/AcOEt 1:1)³⁹). The solvent was evaporated. Flash chromatography (25 g SiO₂, hexane/ Et₂O 2:1) of the residue separated only the excess of Bu₃SnH from the mixture. Prep. HPLC (Zorbax-Sil, hexane/Et₂O 2:1) of the sirupy crude product afforded 100mg (58%) of **87** as the main product. The IR spectra of 3 by-products (small amounts) were not compatible with the structure of the epimer of **87**.

Data of **87**. B.p. 135-140°/10⁻⁴ Torr, $[\alpha]_D^{25} = -21^\circ$ (c = 1.5, CHCl₃). IR: 2995s, 2940s, 2870m, 2250m, 1455m, 1384s, 1374s, 1230s, 1163s, 1111s, 1070s, 1053sh, 998m, 982m, 866m, 842s. ¹H-NMR (200 MHz): 4.82 (dd, J=6.1, 3.5, H-C(6)); 4.68 (dd, J=6.1, 3.8, H-C(5)); 4.48-4.33 (m, H-C(8)); 4.17-3.98 (ddd, AB-part of an ABX-system, H₂-C(9)); 3.72-3.60 (m, H-C(4)); 3.56 (dd, J=7.3, 3.5, H-C(7)); 2.60-2.45 (m, H₂-C(2)); 2.22-1.88 (m, H₂-C(3)); 1.45 (s, CH₃); 1.44 (s, CH₃); 1.37 (s, CH₃); 1.33 (s, CH₃). ¹³C-NMR (25.2 MHz): 118.98(s); 112.10(s); 108.53(s); 81.23(d); 80.68(d); 80.47(d); 79.20(d); 72.67(d); 66.36(t); 26.57(q); 25.39(q); 25.01(q); 24.35(q); 24.22(t); 13.86(t). EI-MS: 282 (22), 239 (1), 224 (4), 164 (7), 123 (11), 101 (47), 95 (16), 59 (19), 43 (100). Anal. calc. for C₁₅H₂₃NO₅ (297.36): C 60.59, H 7.80, N 4.71; found: 60.35, H 7.68, N 4.69.

6-Acetoxy- 7,10-anhydro-1,2:3,4:8,9:11,12-tetra- O-isopropylidene- α -D-erythro-L-manno-D-galacto-dodeco-1,5-pyranose (**89**). A solution of 230 μ l (0.87 mmol) of Bu₃SnH in 0.5 ml of benzene was added dropwise to a solution of 100 mg (0.17 mmol) of **88** [122] and 12 mg (0.07 mmol) of AIBN in 3.4 ml of benzene. The mixture was kept under reflux for 10 h until **88** had disappeared (TLC, CHCl₃/AcOEt 9:1). After evaporation of the solvent, the residue was purified by flash chromatography (15 g SiO₂, hexane/Et₂O 6:4) yielding 85 mg (89%) of **89** as a colourless foam, m.p. 174-175° (dec.), $[\alpha]_D^{25} = -45^\circ$ (c = 1.2, CHCl₃). IR: 2990s, 2935m, 1745s, 1452w, 1382s, 1370s, 1225s, 1166s, 1095s, 1070s, 1045sh, 1005s. ¹H-NMR (200 MHz): 5.71 (dd, J=9.3, 4.9, H-C(6)); 5.49 (d, J=5.1, H-C(1)); 4.89 (dd, J=6.4, 3.7,

³⁹) Detection: spraying with a vanillin reagent (1 g vanillin, 400 ml MeOH and 100 ml 50% aq. H₂SO₄), followed by heating to about 200°.

H-C(8)); 4.70 (dd, $J=6.4, 3.8$, H-C(9)); 4.61 (dd, $J=8.0, 2.5$, H-C(3)); 4.40-4.16 (m, H-C(2), H-C(4), H-C(5), H-C(11)); 4.11-3.95 (ddd, AB-part of an ABX-system, H₂-C(12)); 3.77 (dd, $J=4.9, 3.7$, H-C(7)); 3.44 (dd, $J=6.7, 3.8$, H-C(10)); 2.03 (s, CH₃); 1.57 (s, CH₃); 1.44 (s, CH₃); 1.40 (s, 2 CH₃); 1.35 (s, CH₃); 1.32 (s, CH₃); 1.30 (s, CH₃); 1.25 (s, CH₃). ¹³C-NMR (25.5 MHz): 169.21(s); 112.37(s); 109.54(s); 108.86(s); 96.43(d); 81.30(d); 80.32(d); 80.15(d); 79.72(d); 73.29(d); 70.56(d); 70.06(d); 66.66(t); 66.58(d); 26.60(q); 25.91(q); 25.75(q); 25.55(q); 25.47(q); 24.91(q); 24.56(q); 24.23(q); 20.83(q). EI-MS: 544 (≤ 1), 529 (29), 486 (1), 471 (2), 443 (2), 293 (5), 251 (5), 235 (5), 193 (8), 151 (6), 141 (6), 129 (6), 113 (18), 101 (60), 100 (38), 85 (17), 71 (17), 59 (28), 43 (100). Anal. calc for C₂₆H₄₀O₁₂ (544.60): C 57.34, H 7.40; found: C 57.43, H 7.14.

1-O-Acetyl-2,5-anhydro-3,4-O-isopropylidene-6-O-trityl-D-allitol (93) and -D-altritol (94). A) From 91. Denitration of 800 mg (1.5 mmol) of 91 [122] afforded after 6 h (TLC, toluene/AcOEt 15:1) and purification by flash chromatography (80 g SiO₂, hexane/Et₂O 4:1) 656 mg (90%) of 93/94 as a colourless oil, slowly crystallizing from a concentrated Et₂O solution, m.p. 92-100°. Anal. HPLC (Zorbax-ODS, MeOH/H₂O/acetonitrile 1:34:65, 50°, $k'=3.8$ and 4.1) indicated a ratio of 46:54. ¹H-NMR (90 MHz): 7.65-7.10 (m, 15 arom. H); 4.95-4.05 (m, H₂-C(1), H-C(2), H-C(3), H-C(4), H-C(5)); 3.50-3.05 (m, H₂-C(6)); 2.12 (s, 0.55 CH₃); 1.95 (s, 0.45 CH₃); 1.55, 1.49 (2s, CH₃); 1.35, 1.32 (2s, CH₃).

B) From 92. Denitration of 40 mg (0.075 mmol) of 92 [122] afforded after 8 h and flash chromatography (15 g SiO₂, hexane/Et₂O 4:1) 32 mg (87%) of 93/94 in a ratio of 48:52 (¹H-NMR).

1-O-Acetyl-2,5-anhydro-3,4-O-isopropylidene-D-allitol (95) and -D-altritol (96). According to [158] a solution of 290 mg (0.59 mmol) of a mixture of 93/94 in 70 ml of 1% MeOH in CH₂Cl₂ was treated with 12 mg of anh. FeCl₃. After 2 h at r.t. 0.5 g of Na₂CO₃ x 10 H₂O was added, the mixture was filtered and the solvent evaporated. Flash chromatography (45 g, SiO₂, hexane/AcOEt 6:4) of the residue gave 48 mg (34%) of 95 and 65 mg (44%) of 96 as colourless oils.

Data of 95. $[\alpha]_D^{25} = -6.2^\circ$ ($c = 1.0$, CHCl_3). IR: 3605w, 3030sh, 2990m, 2935m, 2880sh, 1742s, 1453w, 1383s, 1372s, 1220s, 1157m, 1116m, 1078s, 1041s, 862m. $^1\text{H-NMR}$ (90 MHz): 4.70 (dd, $J=7$, 4, H-C(1)); 4.52 (dd, $J=7$, 3, H-C(1)); 4.35-4.00 (m, 4 H); 3.80 (dd, $J=12$, 3, H-C(6)); 3.60 (dd, $J=12$, 4, H-C(6)); 2.43 (br., OH); 2.10 (s, CH_3); 1.54 (s, CH_3); 1.34 (s, CH_3). Anal. calc. for $\text{C}_{11}\text{H}_{18}\text{O}_6$ (246.26): C 53.65, H 7.37; found: C 53.60, H 7.62.

Data of 96. $[\alpha]_D^{25} = +10.8^\circ$ ($c = 1.1$, CHCl_3). IR: 3630w, 3030sh, 2998m, 2942m, 2880sh, 1740s, 1456w, 1386s, 1375s, 1230s, 1165m, 1122s, 1078s, 1040s, 875m. $^1\text{H-NMR}$ (90 MHz): 4.90-4.00 (m, 6 H); 3.67 (t, $J=4.5$, $\text{H}_2\text{-C}(6)$); 2.22 (br., OH); 2.10 (s, CH_3); 1.48 (s, CH_3); 1.33 (s, CH_3). Anal. calc. for $\text{C}_{11}\text{H}_{18}\text{O}_6$ (246.26): C 53.65, H 7.37; found: C 53.84, H 7.44.

1,6-Di-O-acetyl-2,5-anhydro-3,4-O-isopropylidene-D-allitol (97). Acetylation of 48 mg (0.20 mmol) of 95 gave after chromatography (10 g SiO_2 , hexane/ Et_2O 2:1) 42 mg (75%) of 97, b.p. $100^\circ/10^{-4}$ Torr. IR: 3025sh, 2985w, 2925w, 2895sh, 1742s, 1450w, 1381m, 1370m, 1220s, 1155m, 1080s, 1040s, 862m. $^1\text{H-NMR}$ (90 MHz): 4.55 (br.s, H-C(3), H-C(4)); 4.45-3.95 (m, $\text{H}_2\text{-C}(1)$, H-C(2), H-C(5), $\text{H}_2\text{-C}(6)$); 2.10 (s, 2 CH_3); 1.55 (s, CH_3); 1.35 (s, CH_3). $^{13}\text{C-NMR}$ (25.2 MHz): 170.34(s); 114.43(s); 82.27(d); 81.90(d); 64.25(t); 27.37(q); 25.48(q); 20.77(q). EI-MS: 273 (24), 153 (25), 111 (12), 69 (15), 68 (15), 59 (11), 43 (100). Anal. calc. for $\text{C}_{13}\text{H}_{20}\text{O}_7$ (288.30): C 54.16, H 6.99; found: C 53.89, H 7.16.

1,6-Di-O-acetyl-2,5-anhydro-3,4-O-isopropylidene-D-altritol (98) [157]. Acetylation of 65 mg (0.26 mmol) of 96 gave after chromatography (15 g SiO_2 , hexane/ Et_2O 2:1) 72 mg (96%) 98, b.p. $115\text{-}120^\circ/10^{-4}$ Torr. $[\alpha]_D^{25} = +1.6^\circ$ ($c = 1.2$, CHCl_3); ([157]: $+3^\circ$ (CHCl_3)). $^1\text{H-NMR}$: same as in [157].

3. 1-C-Nitroglycals: Synthesis and Reaction with Nitrogen Nucleophiles

D-Mannose-oxime (113). To a soln. of hydroxylamine-hydrochloride (9.2 g, 133 mmol; dried over P_2O_5 for 24 h) in 95% EtOH (250 ml) was added a soln. of Na (3.1 g, 133 mmol) in 95% EtOH (250 ml). The mixture was heated to 60° (30 min), cooled to 0° and filtered. The filtrate was added to a soln. of D-mannose (112); 20 g, 111 mmol) at 60° and stirred at this temperature for 30 min. A precipitate appeared and stirring was continued over night. TLC (AcOEt/MeOH/H₂O 7:2:1) indicated the disappearance of 112. The mixture was concentrated, cooled and the crystals were collected and dried giving 113 (220 g, quant.). M.p. 182-183° ([279]: 176-184°).

N-(p-Nitrobenzylidene)-β-D-mannopyranosylamine-N-oxide (114). A suspension of the oxime 113 (30 g, 154 mmol), p-nitrobenzaldehyde (30 g, 198 mmol) and p-TsOHxH₂O (285 mg) in dry DMSO (150 ml) was three times degassed by evaporation. The mixture was stirred under an N₂ atmosphere until a clear yellow soln. was formed (90 min). Although TLC (AcOEt/MeOH/H₂O 7:2:1) showed traces of 113, the solvent was distilled (45°, 10⁻³ mbar) and the honey-like residue was dissolved in anh. MeOH (60 ml), from which the nitrone 114 precipitated. The mixture was diluted with AcOEt (150 ml) and stored over night in the refrigerator⁴⁰). The crystals were collected, washed with AcOEt and dried (24 h over P_2O_5) giving the nitrone 114 (45.8 g, 91%). M.p. 98-100° (dec.). $[\alpha]_D^{25} = +107.3^\circ$ (c = 1.1, DMSO; no mutarotation was observed). IR (KBr): 3360s(br.), 1600s, 1570s, 1510s, 1426m, 1350s, 1310s, 1250w, 1160s, 1110s, 1095s, 1080s, 1038s, 1015s, 958m, 870m, 792m, 705m. ¹H-NMR (200 MHz, (D₆)DMSO): 8.59 (d, J=9.3, 2 H arom.); 8.33 (d, J=9.3, 2 H arom.); 8.16 (s, 1 H); 5.30-4.36 (m(br.), 4 x OH); 5.07 (s, H-C(1)); 3.88-3.30 (m, H-C(2), H-C(3), H-C(4), H-C(5), 2 H-C(6)). ¹³C-NMR (50 MHz, (D₆)DMSO): 147.04(s); 136.04(s); 129.92(d); 129.24(d); 123.60(d); 95.28(d); 79.79(d); 73.43(d); 68.62(d); 66.22(d); 60.96(t). FAB-MS: 329 ([M + 1]⁺). Anal. calc. for C₁₃H₁₆N₂O₈ x H₂O

⁴⁰) Without placing the mixture overnight in the refrigerator lower yields were obtained.

(346.30): C 45.09, H 5.24, N 8.09; found: C 45.20, H 5.38, N 7.88.

1-Deoxy-1-nitro-D-mannopyranose (115). A soln. of the nitrone 114 (15.0 g, 46 mmol) in MeOH (99%, 350 ml) was cooled to -70° and ozonized until TLC (AcOEt/MeOH/H₂O 7:2:1) indicated the disappearance of 114. The mixture was purged with N₂, treated with dimethylsulfide (3.3 ml) and warmed to r.t. MeOH was removed (at 20°) and the residue was dissolved in H₂O (200 ml) and extracted with AcOEt. The water layer was freeze dried and the oily residue was stored under anh. MeOH forming a slightly yellow solid, which was dried over P₂O₅ (8.2 g). A sample of 4 g of this solid was crystallized from hot AcOEt (40 ml) and anh. MeOH (7 ml) giving 1-deoxy-1-nitro-D-mannose 115 (3.1 g). M.p. $100-102^{\circ}$ (dec.). ¹H-NMR (90 MHz, D₂O): 5.70 (s(br.), H-C(1)); 4.52 (d(br.), J=3.0, H-C(2)); 3.95-3.38 (m, H-C(3), H-C(4), H-C(5), 2 H-C(6)). ¹³C-NMR (25.2 MHz, D₂O): 102.80(d); 78.96(d); 72.67(d); 70.69(d); 66.13(d); 60.91(t).

1-Deoxy-4,6-O-isopropylidene-1-nitro- α - and - β -D-mannopyranose (116 and 117). A soln. of the nitrone 114 (20.1 g, 61.2 mmol) in 90% EtOH (600 ml) and DMF (5 ml) at -40° was ozonized until TLC (AcOEt/MeOH/H₂O 85:12:3) indicated the disappearance of 114. The soln. was purged with N₂, dimethylsulfide (4.4 ml) was added, and then worked up as described for 115. The crude 1-deoxy-1-nitro-mannose 115, obtained as a colourless foam, was dissolved in DMF (200 ml) and cooled to -40° . After addn. of p-TsOH.H₂O (120 mg) 2-methoxypropene (11.6 ml, 123 mmol) was added and the mixture was stored at -30° (20 h). Additional 2-methoxypropene (11.6 ml) and p-TsOH.H₂O (150 mg) was added and the soln. was stored at -30° until TLC (AcOEt/hexane 7:3) indicated the disappearance of 5 (62 h). After the excess of p-TsOH.H₂O has been neutralized with NaHCO₃ (120 mg) the solvent and excess of 2-methoxypropene were removed at 10^{-3} mbar. The residue was dissolved in pyridine/AcOEt/hexane 2:2:1 and flash chromatographed (hexane/AcOEt 3:7) on a preconditioned column (300 g SiO₂, hexane/AcOEt 6:4). Upon concentration of the pooled fractions, pure 116 crystallized. The mother liquor was evaporated to dryness to give an anomeric mixture of 116/117 as a colourless solid (total yield 66%).

Data of 116. M.p. 163-164° (dec.). $[\alpha]_D^{25} = +10.50^\circ$ (c = 1.25, MeOH). IR (KBr): 3490s, 3375s, 3000m, 2925m, 1560s, 1540m, 1375s, 1264m, 1196s, 1175s, 1122s, 1102s, 1065s, 1046s, 1010m, 950m, 901m, 882m, 854s, 810s. $^1\text{H-NMR}$ (200 MHz, CD_3OD): 5.66 (d, J=1.7, H-C(1)); 4.52 (dd, J=3.2, 1.7, H-C(2)); 4.06-3.94 (m, 3H); 3.70 (dd, J=9.8, 3.2); 3.44 (ddd, J=9.5, 8.8, 6.3, H-C(5)); 1.56 (s, CH_3); 1.41(s, CH_3). $^{13}\text{C-NMR}$ (25.2 MHz, $\text{CDCl}_3/(\text{D}_6)\text{DMSO}$): 101.80(d); 98.80(s); 69.89(d); 69.53(d); 68.80(d); 68.71(d); 60.39(t); 28.09(q); 18.23(q).

Data of 117. $^{13}\text{C-NMR}$ (25.2 MHz, $\text{CDCl}_3/(\text{D}_6)\text{DMSO}$): 105.39(d); 69.11(d); 69.02(d); 68.28(d); 66.85(d).

3-O-acetyl-1,2-dideoxy-4,6-O-isopropylidene-1-nitro-D-arabino-hex-1-enopyranose (118). To a suspension of an mixture of 116/117 (5.0 g, 20.1 mmol) in Et_2O (50 ml) at 0° was added (1 h) a soln. of Ac_2O (7.5 ml) and pyridine (16.2 ml) in Et_2O (30 ml). After 4 h TLC ($\text{AcOEt}/\text{hexane}$ 7:3) indicated the disappearance of 116/117. The soln. was cooled to 0°, 10% aq. NaHCO_3 (100ml) was added and the mixture was stirred vigorously for 10 min. After dilution with CH_2Cl_2 , the mixture was extracted twice with 5% aq. NaHCO_3 , the organic layer was concentrated, and dissolved in CH_2Cl_2 (100 ml). The soln. was treated with Amberlite IRA-93 (5 g) at r.t. over night. The resin was filtered off and the filtrate was evaporated. FC (200 g SiO_2 , $\text{hexane}/\text{AcOEt}$ 7:3) gave the nitro-olefin 118 (4.1 g, 75%). For analysis, a sample was crystallized from $\text{Et}_2\text{O}/\text{hexane}$. M.p. 97-98°. $[\alpha]_D^{25} = -134^\circ$ (c = 1.4, CHCl_3). UV (CH_2Cl_2): 285 (3690). IR: 3110w, 3000w, 2890w, 1745s, 1670m, 1550s, 1378s, 1344s, 1215s(br), 1125s, 1100s, 1028s, 925w, 860m. $^1\text{H-NMR}$ (200 MHz): 6.26 (d, J=2.5, H-C(2)); 5.59 (ddd, J=5.0, 2.5, H-C(3)); 4.28-3.97 (m, H-C(4), H-C(5), 2 H-C(6)); 2.14 (s, CH_3); 1.55(s, CH_3); 1.44(s, CH_3). $^{13}\text{C-NMR}$ (25.2 MHz): 170.06(s); 153.35(s); 100.55(d); 100.33(s); 72.46(d); 68.22(d); 68.17(d); 60.81(t); 28.54(q); 20.86(q); 18.87(q). CI-MS: 274 ($[\text{M} + 1]^+$, 100). Anal. calc. for $\text{C}_{11}\text{H}_{15}\text{NO}_7$ (273.24): C 48.35, H 5.53, N 5.13; found: C 48.40, H 5.60, N 5.00.

4,6-O-benzylidene-1,2-dideoxy-1-nitro-D-arabino-hex-1-enopyranose **123** and 4,6-O-benzylidene-1-deoxy-2-O-methyl-1-nitro- α - and - β -D-mannopyranoses (**124/125**). A) From **121**. To a soln. of **121** (1.0 g, 3.11 mmol) in THF (15 ml) and anh. MeOH (10 ml) at -20° was added (through a teflon tube) a soln. of NaOMe/MeOH (36 mg (1.56 mmol) Na in 20 ml anh. MeOH) at -20° . After 2.5 h at -20° , TLC (AcOEt/hexane 4:6) showed traces of **121** only. The mixture was neutralized with AcOH (90 μ l, 1.56 mmol), stirred at -20° (20 min), diluted with CH_2Cl_2 (50 ml) and extracted with H_2O , 5% aq. NaHCO_3 and brine to give after removal of the solvent crude **123** (920 mg). Crystallization from AcOEt/hexane gave **123** (638 mg, 74%). FC (25 g SiO_2 , hexane/AcOEt 25:9) of the mother liquor yielded **121** (14 mg, 1.4%), **123** (91 mg, 10%) and a mixture of **124/125** (38 mg, 4%)⁴¹. An analytical sample of **15** was obtained by recrystallization from AcOEt/hexane.

Data of **123**. M.p. $173-174^{\circ}$. $[\alpha]_{\text{D}}^{25} = -12.2^{\circ}$ ($c = 0.8$, CHCl_3). UV (CH_2Cl_2): 287 (3380). IR: 3595m, 3100w, 2870m, 1665m, 1550s, 1465w, 1450w, 1375mn, 1345s, 1280m, 1140s, 1100s, 1085s, 1025m, 905m. $^1\text{H-NMR}$ (200 MHz, CDCl_3): 7.55-7.29 (m, 5 H arom.); 6.28 (d, $J=2.8$, H-C(2)); 5.62 (s, ArCH); 4.72 (dd, $J=7.8$, 2.8, H-C(3)); 4.57 (dd, $J=10.3$, 4.9, H-C(6)); 4.20 (ddd, $J=10.3$, 10.0, 4.9, H-C(5)); 3.99 (dd, $J=10.3$, 10.3, H-C(6)); 3.88 (dd, $J=10.0$, 7.8, H-C(4)); 2.60 (s(br.), OH). $^{13}\text{C-NMR}$ (25.2 MHz): 151.6(s); 136.9(s); 128.9(d); 127.9(d); 126.1(d); 106.3(d); 100.7(d); 77.8(d); 70.8(d); 66.5(t); 65.0(d). CI-MS: 280 ($[\text{M} + 1]^+$, 100). Anal. calc. for $\text{C}_{13}\text{H}_{13}\text{NO}_6$ (279.25): C 55.92, H 4.69, N 5.02; found: C 55.65, H 4.70, N 4.84.

Data of **124/125**. M.p. $192-193^{\circ}$. $[\alpha]_{\text{D}}^{25} = +34.9^{\circ}$ ($c = 1.1$, acetone). IR(KBr): 3370m, 3310(sh), 2945w, 2870w, 1560w, 1472s, 1456w, 1388m, 1372m, 1218m, 1198m, 1186m, 1162m, 1120s, 1102s, 1055s, 1042m, 986s, 975s. $^1\text{H-NMR}$ (400 MHz, (D_6)acetone): 7.51-7.33 (m, 5 arom. H); 6.01 (d, $J=1.5$, 0.85 H-C(1)); 5.82 (d, $J=1.7$, 0.15 H-C(1)); 5.66 (s, ArCH); 4.86 (d, $J=5.8$, 0.15 OH); 4.56 (d, $J=6.8$, 0.85 OH); 4.38 (dd, $J=3.3$, 1.7, 0.15 H-C(2)); 4.32 (dd, $J=3.5$, 1.5, 0.85 H-C(2)); 4.31 (dd, $J=9.5$, 4.0,

⁴¹) Larger batches (15-20 g of **121**) gave **123** in yields of 80-82%.

H-C(6)); 4.16 (ddd, J=9.8, 5.8, 3.3, 0.15 H-C(3)); 4.01 (dd, J=9.5, 9.5, 0.85 H-C(4)); 3.91 (ddd, J=10.0, 9.5, 4.0, 0.85 H-C(5)); 3.85 (dd, J=10.0, 9.5, H-C(6)); 3.81 (ddd, J=9.5, 6.8, 3.3, 0.85 H-C(3)); 3.62 (s, 0.85 CH₃); 3.59 (ddd, J=10.0, 9.5, 4.6, 0.15 H-C(5)); 3.51 (s, 0.15 CH₃). ¹³C-NMR (50.4 MHz, (D₆)DMSO): 137.5(s); 137.4(s); 129.0(d); 128.0(d); 126.4(d); 103.5(d); 101.6(d); 101.1(d); 80.8(d); 80.0(d); 77.3(d); 76.5(d); 70.3(d); 69.0(d); 68.1(d); 67.2(d); 67.0(t); 61.8(q); 59.5(q). CI-MS: 312 ([M + 1]⁺). Anal. calc. for C₁₄H₁₇NO₇ (311.30): C 54.02, H 5.50, N 4.50; found: C 54.30, H 5.75, N 4.23.

B) From 127. To an ice-cold soln. of 127 (420 mg, 1.37 mmol) in THF (15 ml) and anh. MeOH (6 ml) was added a 0.5 M NaOH/MeOH soln. (50 µl). After 10 min, 127 had disappeared as indicated by TLC (hexane/AcOEt 6:4). The mixture was poured onto ice water (20 ml) and extracted with AcOEt. The solvent was removed and 123 (238 mg, 62%) was crystallized from AcOEt/hexane. M.p. 173-174°. FC (25 g SiO₂, AcOEt/hexane 9:25) gave further 123 (76 mg, 20%).

C) From 129. A suspension of 129 (9.5 g, 26 mmol) in MeOH (240 ml) was cooled to 0° and treated with sodium 2-nitrophenolate (430 mg, 2.6 mmol). After 30 min, additional sodium 2-nitrophenolate (215 mg, 1.3 mmol) was added⁴²⁾⁴³⁾. When the solution was homogeneous and TLC (hexane/AcOEt 6:4) indicated the disappearance of 129, the solvent was removed at 10-20°⁴⁴⁾. The residue was dissolved in AcOEt/hexane 3:1, SiO₂ (16 g) was added and the mixture was stirred at r.t. for 30 min.. SiO₂ was filtered off, washed with AcOEt/hexane 3:1. The filtrate was taken to dryness and the residue was crystallized from AcOEt/hexane to give 123 (5.73 g, 79%). FC (100 g of SiO₂, hexane/AcOEt 5:2) of the mother liquor gave further

42) Smaller batches did not require additional sodium 2-nitrophenolate.

43) Shorter reaction times led usually to cleaner reactions and to better yields.

44) Higher temperatures gave by-products.

123 (1.04 g, 14%). M.p. 173-174°. $[\alpha]_D^{25}$ and $^1\text{H-NMR}$ were in accordance with the published data.

4,6-O-Benzylidene-1,2-dideoxy-3-O-formyl-1-nitro-D-arabino-hex-1-eno-
pyranose (127). To an ice-cold soln. of 120 (500 mg, 1.68 mmol) in CH_2Cl_2 (10 ml) was added a 2:5 mixture of $\text{Ac}_2\text{O}/\text{HCOOH}^{45}$ (10 ml) [174][175]. After 4h at 0°, TLC ($\text{AcOEt}/\text{hexane}/\text{CHCl}_3$ 2:2:1) indicated the disappearance of 120. The mixture was concentrated, diluted with CH_2Cl_2 (100 ml) and extracted with sat. NaHCO_3 , 5% NaHCO_3 and brine to give after removing of the solvent the crude formylated 1-deoxy-1-nitro-D-glucose 126, which was dissolved in CHCl_3 (5 ml) and treated with Amberlite IRA-93 (3 g). The mixture was stirred at 60° until TLC indicated completion of the reaction (5 h). The resin was filtered off, washed with CHCl_3 (3 ml, 30 min.) and the filtrates were concentrated. FC (50 g of SiO_2 , $\text{Et}_2\text{O}/\text{hexane}$ 3:7) gave 127 (430 mg, 83%). An anal. sample was obtained by crystallisation from $\text{AcOEt}/\text{hexane}$. M.p. 119-120°. $[\alpha]_D^{25} = -90.0^\circ$ ($c = 1.0$, CHCl_3). UV (CH_2Cl_2): $\lambda_{\text{max}} = 282$ (3550). IR: 3100w, 3020w, 2930w, 2870w, 1730s, 1665m, 1548s, 1368m, 1340s, 13330s, 1278m, 1132s, 1090s, 1024s. $^1\text{H-NMR}$ (200 MHz): 8.14 (d, $J=1.1$, HCOO-); 7.56-7.36 (m, 5 arom. H); 6.31 (d, $J=2.7$, $\text{H-C}(2)$); 5.90 (ddd, $J=8.0$, 2.7, 1.1, $\text{H-C}(3)$); 5.63 (s, ArCH); 4.62 (dd, $J=10.5$, 5.0, $\text{H-C}(6)$); 4.33 (ddd, $J=10.0$, 10.0, 5.0, $\text{H-C}(5)$); 4.16 (dd, $J=10.0$, 8.0, $\text{H-C}(4)$); 4.05 (dd, $J=10.5$, 10.0, $\text{H-C}(6)$). $^{13}\text{C-NMR}$ (50 MHz): 159.86(d); 153.68(s); 135.91(s); 129.56(d); 128.40(d); 126.08(d); 101.93(d); 99.74(d); 75.15(d); 71.32(d); 67.48(t); 67.20(d). CI-MS: 308 ($[\text{M} + 1]^+$), 262, 233, 107, 105, 73. Anal. calc. for $\text{C}_{14}\text{H}_{13}\text{NO}_7$ (307.24): C 54.73, H 4.26, N 4.56%; found: C 54.87, H 4.13, N 4.39.

4,6-O-Benzylidene-3-O-chloroacetyl-1,2-dideoxy-1-nitro-D-arabino-hex-1-enopyranose (129). To an ice-cold soln. of 120 (16.0 g, 54 mmol) in dry ether (100 ml) was added dropwise (1 h) a soln. of chloroacetic acid anhydride (35 g, 204 mmol) and pyridine (43.5 ml) in dry ether (200

45) The mixture was prepared 4 h in advance.

46) $\text{ClCH}_2\text{CO})_2\text{O}$ was first dissolved in ether and then pyridine was added.

ml)⁴⁶). After 1.5 h at 0°, TLC (AcOEt/hexane/CHCl₃ 2:2:1) indicated the disappearance of 120. Excess chloroacetic acid anhydride was destroyed by adding dropwise a soln. of sat. NaHCO₃ (200 ml) at 0°. The two layers were separated, the organic layer was diluted with ether (200 ml) and extracted with 5% NaHCO₃ (200 ml), sat. aq. CuSO₄ (2 x 200 ml), 5% NaHCO₃ (200 ml) and brine (200 ml). The aq. layers were washed with ether (300 ml). The combined organic layers were evaporated and dried (for 2 h at 10⁻² mbar) to give crude 128 (24 g, 99%). To a suspension of crude 128 (22 g, 49 mmol) in benzene (400 ml) was added Amerlite IRA-93 (44 g). The mixture was warmed to 60° and stirred until TLC (hexane/AcOEt/CH₂Cl₂ 5:2:1) indicated the disappearance of 128. The resin was filtered off, washed with CH₂Cl₂ (20 ml, 30 min.), the filtrate and washing were combined and extracted with 10% NaHCO₃ (2 x 300 ml) and brine. The solvent was removed to give crude 129 (13.4 g, 77%) as a yellow-brown solid. Crystallisation from CH₂Cl₂/AcOEt/hexane gave pure 129 (8.4 g, 48%). FC (400 g of SiO₂, hexane/CH₂Cl₂/AcOEt 5:2:1) of the mother liquor gave further 129 (1.1 g, 6.5%). An anal. sample was obtained by recrystallisation from AcOEt/hexane. M.p. 150-151°. $[\alpha]_D^{25} = -111.6^\circ$ (c = 1.1, CHCl₃). UV (CH₂Cl₂): $\lambda_{\max} = 281$ (3560). IR: 3100w, 3020w, 2935w, 2870w, 1765s, 1748sh, 1668m, 1548s, 1369m, 1344m, 1331s, 1278s, 1170s, 1130s, 1098s, 1089s, 1023m, 1000s. ¹H-NMR (200 MHz): 7.54-7.34 (m, 5 arom. H); 6.31 (d, J=2.7, H-C(2)); 5.84 (dd, J=7.5, 2.7, H-C(3)); 5.63 (s, ArCH); 4.62 (dd, J=10.5, 5.0, H-C(6)); 4.32 (ddd, J=10.0, 10.0, 5.0, H-C(5)); 4.16 (dd, J=10.0, 7.5, H-C(4)); 4.14 (s, CH₂Cl); 4.05 (dd, J=10.5, 10.0, H-C(6)). ¹³C-NMR (50 MHz): 166.74(s); 153.72(s); 135.88(s); 129.58(d); 128.40(d); 126.09(d); 101.93(d); 99.44(d); 75.02(d); 71.29(d); 69.28(d); 67.46(t); 40.46(t). CI-MS: 358 ([M + 3]⁺, 37%) and 356 ([M + 1]⁺, 100%). Anal. calc. for C₁₅H₁₄ClNO₇ (355.74): C 50.65, H 3.97, Cl 9.96, N 3.94; found: C 50.68, H 4.05, Cl 10.16, N 3.82.

2-Acetamido-4,6-O-benzylidene-1,2-dideoxy-1-nitro- α - and - β -D-mannopyranose (130/131). To a soln. of 121 (10.0 g, 31.1 mmol) in THF (200 ml) was added (8 h) a soln. of NH₃ (25% in H₂O, 23 ml). After 24 h, TLC (AcOEt) indicated the disappearance of 121. Removal of the solvent and flash chromatography (200 g SiO₂, AcOEt/hexane 4:1) of the residue gave a mixture of 130/131 (85:15, 8.40 g, 80%) as a colourless solid. Crystallisation from AcOEt/hexane gave a pure sample of 130.

Data of 130. M.p. 121-122° (dec.) $[\alpha]_D^{25} = +45.4^\circ$ (c = 1.0, DMSO). IR(KBr): 3405s, 2915w, 2870w, 1670s, 1565s, 1515s, 1376s, 1180m, 1113s, 990m, 754m, 702m. $^1\text{H-NMR}$ (400 MHz, $(\text{D}_6)\text{DMSO}$): 8.28 (d, J=8.0, NH); 7.55-7.33(m, 5 h arom.); 5.86 (d, J=1.0, H-C(1)); 5.59 (s, ArCH); 5.10 (ddd, J=8.0, 5.0, 1.0, H-C(2)); 4.31 (dd, J=9.8, 4.6, H-C(6)); 4.08 (dd, J=9.8, 9.8, H-C(4)); 3.81 (dd, 9.8, 9.8, H-C(6)); 3.73 (dd, J=9.8, 5.0, H-C(3)); 3.72 (ddd, J=9.8, 9.8, 4.6, H-C(5)); 1.98 (s, CH_3). $^{13}\text{C-NMR}$ (50 MHz, $(\text{D}_6)\text{DMSO}$): 170.1(s); 137.2(s); 128.9(d); 127.9(d); 126.2(d); 105.2(d); 101.3(d); 76.3(d); 68.8(d); 67.2(t); 65.0(d); 51.5(d); 22.6(q). Anal. calc. for $\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_7$ (338.08): C 53.25, H 5.32, N 8.28; found: C 53.03, H 5.24, N 8.41.

Data of 131. $^1\text{H-NMR}$ (200 MHz, $(\text{D}_6)\text{DMSO}$): 7.98 (d, J=9.0, NH); 7.50-7.38 (m, 5 H arom.); 6.08 (d, J=2.2, H-C(1)); 5.58 (s, ArCH); 5.53 (d, J=5.4, OH); 5.08-4.98 (m, 1 H); 4.34 (dd, J=4.8, 4.6, 1 H); 4.10-3.84 (m, 4 H); 3.62 (s(br.), 1 H); 1.85 (s, CH_3). $^{13}\text{C-NMR}$ (50 MHz, $(\text{D}_6)\text{DMSO}$): 170.1(s); 137.3(s); 129.1(d); 128.1(d); 126.4(d); 105.3(d)?; 101.3(d); 76.3(d); 68.8(d); 67.1(t); 64.9(d); 51.4(d); 22.6(q).

2-Acetamido-1,2-dideoxy-4,6-O-isopropylidene-1-nitro- α - and - β -D-manno pyranose (132/133). To an ice-cold soln. of 118 (4.1 g, 15 mmol) in THF (410 ml) was added a soln of NH_3 (25% in H_2O , 53 ml). The flask was stoppered and the mixture was stirred at r.t. for 36 h. When TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 15:1) had indicated the disappearance of 118. The solvent was removed and the residue was dried (1 h, 10^{-2} mbar). FC (200 g SiO_2 , $\text{MeOH}/\text{CH}_2\text{Cl}_2$ 1:29) of the residue gave a mixture of 132/133 (85:15, 4.0 g, 93%) as colourless crystals. M.p. 140°(dec.). $[\alpha]_D^{25} = +65.7^\circ$ (c = 1.2, acetone). IR(KBr): 3300(br.), 3005w, 1667s, 1646s, 1568s, 1545s, 1462m, 1448m, 1373m, 1290w, 1270m, 1220m, 1182s, 1128s, 1097s, 1057m, 860s, 742m. $^1\text{H-NMR}$ (200 MHz, $(\text{D}_6)\text{DMSO}$): 8.19 (d, J=8.5, 0.85 NH); 7.90 (d, J=10.0, 0.15 NH); 5.99 (s(br.), 0.15 H-C(1)); 5.83 (s, 0.85 H-C(1)); 5.45 (d, J=5.0, 0.85 OH); 5.35 (d, J=5.5, 0.15 OH); 5.12 (dd, J=8.5, 5.0, 0.85 H-C(2)); 4.97 (d(br.), J=10.0, 0.15 H-C(2)); 4.10 (dd, J=10.0, 10.0, 0.85 H-C(4)); 4.00-4.30 (m, H-C(3), 0.15 H-C(4), H-C(5), 2 H-C(6)); 1.95 (s, 0.85 CH_3); 1.84 (s, 0.15 CH_3); 1.50 (s, CH_3); 1.32 (s, CH_3). $^{13}\text{C-NMR}$ (25.2 MHz, $(\text{D}_6)\text{DMSO}$): mainproduct: 169.80(s); 105.44(d); 99.63(s);

69.91(d); 69.00(d); 65.27(d), 60.78(t); 51.18(d); 28.92(q); 22.68(q); 19.56(q); minorproduct: 169.69(s); 101.72(d); 99.63(s); 69.71(d); 68.13(d); 52.49(d). EI-MS: 275 (2, $M^+ - 15$), 244 (1, $M^+ - NO_2$), 243 (8, $M^+ - HNO_2$), 228 (8). Anal. calc. for $C_{11}H_{18}N_2O_7$ (290.27): C 45.52, H 6.25, N 9.65; found: C 45.59, H 6.39, N 9.51.

4. Synthesis of N-Acetylneuraminic Acid and N-Acetyl-4-epineuraminic Acid

tert-Butyl 5-Acetamido-7,9-O-benzylidene-2,3,5-trideoxy-2-methylidene-D-manno-4-nonulosonate (143) and tert-Butyl 5-Acetamido-7,9-O-benzylidene-2,3,5-trideoxy-2-methylidene-D-manno-4-nonulopyranosonate(144). To an ice cold soln. of 2-Acetamido-4,6-O-benzylidene-1,2-dideoxy-1-nitro- D-man-nopyranose (130/131) (8.40 g, 24.8 mmol) and tert-butyl 2-(bromomethyl)prop-2-enoate (54) (8.40 g, 37.2 mmol) in THF (75 ml) was added dropwise a soln. of DBU (8.40 g, 49.6 mmol) in THF (25 ml) within 4 h. Stirring of the mixture was continued at 0° for 20 h. The precipitate was filtered off, and the filtrate was diluted with AcOEt (200 ml). Extrac-tive workup (H₂O, brine) gave the crude product (13.1 g; TLC: CH₂Cl₂/MeOH 92:8), which was redissolved in THF (80 ml) and phosphate buffer pH 6.6 (20 ml) and treated with urea (1.95 g, 27.3 mmol). The mix-ture was stirred at r.t. for 3 days and then diluted with AcOEt (100 ml). Extractive workup (5%-NaHCO₃, H₂O, brine) gave a yellow oil, which was redissolved in AcOEt/Et₂O 1:1. Addition of hexane afforded a precipitate, which was filtered off and crystallized from AcOEt/Et₂O/hexane yielding slightly yellow crystals of 143/144 (2.2 g). FC of the combined mother liquors (500 g SiO₂, CH₂Cl₂/MeOH 97:3) gave further 143/144 (5.0 g, total yield 64%). M.p. 143 - 144°; $[\alpha]_D^{25} = +20.5^\circ$ (c = 1.0, DMSO). IR: 3435m, 3320m (br.), 3000m, 2985m, 2935w, 2870w, 1715 (sh), 1680s, 1626m, 1502m, 1392m, 1370s, 1356m, 1152s, 1090s, 1030m. ¹H-NMR (400 MHz, (D₆)DMSO): 8.24 (d, J=9.0, 0.65 NH); 7.60 (d, J=10.0, 0.35 NH); 7.53-7.27 (m, 5 H arom.); 6.04 (d, J=1.2, 0.65 H olef.); 6.00 (d, J=1.5, 0.35 H olef.); 5.63 (d, J=1.5, 0.35 H olef.); 5.51 (s, 0.35 ArCH); 5.50 (d, J=1.2, 0.65 H olef.); 5.40 (s, 0.65 ArCH); 5.19 (d, J=6.0, 0.65 OH); 4.95 (d, J=8.0, 0.65 OH); 4.73 (dd, J=9.0, 9.0, 0.65 H-C(5)); 4.16 (ddd, J=10.0, 10.0, 5.0, 0.65 H-C(8)); 4.21-3.44 (m, 7.35 H); 1.88 (s, 0.35 CH₃); 1.84 (s, 0.65 CH₃); 1.42 (s, 0.35 t-Bu); 1.37 (s, 0.65 t-Bu). ¹³C-NMR(50 MHz, (D₆)DMSO): 143: 206.07(s); 168.81(s); 165.00(s); 138.15(s); 135.98(s); 128.32(d); 127.70(d); 126.99(t); 126.11(d); 99.77(d), 80.34(d); 80.00(s); 70.88(t); 67.26(d); 59.47(d); 57.12(d); 44.84(t); 27.49(q); 22.36(q); 144: 169.76(s); 166.31(s); 137.87(s); 136.10(s); 128.70(d); 127.89(d); 126.64(t); 126.27(d); 101.20(d); 99.44(s); 79.62(s); 78.66(d); 68.31(t);

66.09(d); 63.89(d); 54.64(d); 37.78(t); 27.63(q); 22.77(q). CI-MS: 450 ($[M + 1]^+$), 432 ($[M + 1]^+ - 18$), 390 ($[M + 1]^+ - 60$), 376, 239, 209, 91. Anal. calc. for $C_{23}H_{31}NO_8$ (449.52): C 61.46, H 6.95, N 3.12; found: C 61.46, H 6.80, N 3.33.

tert-Butyl 5-Acetamido- 2,3,5-trideoxy- 7,9-O-isopropylidene-2-methylidene-D-manno-4-nonulosonate (145) and tert-Butyl 5-Acetamido- 2,3,5-trideoxy- 7,9-O-isopropylidene-2-methylidene-D-manno-4-nonulosonate (146).

To an ice cold soln. of 132/133 (2.0 g, 6.90 mmol) and DBU (2.1 g, 13.80 mmol) in THF (25 ml) tert-butyl 2-(bromomethyl)prop-2-enoate (54) (2.3 g, 10.40 mmol) in THF (5 ml) was added dropwise. After 2 h, TLC ($CH_2Cl_2/MeOH$ 9:1) indicated the disappearance of 132/133. Phosphate buffer pH 6.6 (10 ml) and urea (0.455 g, 7.60 mmol) were added, and the mixture was stirred at r.t. for 3 days, when TLC ($CH_2Cl_2/MeOH$ 9:1) showed only traces of the intermediate nitro compound, the mixture was diluted with AcOEt (100 ml) and extracted with brine (50 ml). Flash chromatography (250 g SiO_2 , $CH_2Cl_2/MeOH$ 20:1) afforded 145/146 (2.3 g, 83%) as a colourless foam. $[\alpha]_D^{25} = -11.4^\circ$ ($c = 1.0$, $CHCl_3$). IR: 3420m (br.), 2980m, 2930w, 2870w, 1720(sh), 1680s, 1632(sh), 1502m, 1370s, 1318m, 1150s, 1064s, 955w, 876m, 856m. 1H -NMR (200 MHz): 6.88 (d, $J=8.0$, 0.65 NH); 6.31 (d, $J=1.0$, 0.65 olef. H); 6.23 (d, $J=1.2$, 0.35 olef. H); 5.84 (d, $J=10.0$, 0.35 NH); 5.66 (d, $J=1.0$, 0.65 olef. H); 5.57 (s (br.), 0.35 olef. H); 5.36 (s, 0.35 OH); 5.00 (dd, $J=8.0$, 5.6, 0.65 H-C(5)); 4.47 (dd, $J=10.0$, 4.5, 0.35 H-C(5)); 4.40-3.54 (m, 0.65 H_2 -C(3), H-C(6) - H_2 -C(9), 0.65 OH); 3.43 (d, $J=8.0$, 0.65 OH); 2.80 (d, $J=3.0$, 0.35 OH); 2.79 and 2.57 (2 x d, $J=14.0$, 0.35 H_2 -C(3)); 2.14 (s, 0.35 CH_3); 2.05 (s, 0.65 CH_3); 1.52-1.40 (5 x CH_3). ^{13}C -NMR (50 MHz, $(D_6)DMSO$): 145: 206.17(s); 168.45(s); 165.08(s); 136.13(s); 127.07(t); 97.92(s); 80.05(d); 72.45(d); 67.31(d); 64.55(t); 60.81(d); 57.12(d); 44.79(t); 28.45(q); 27.55(q); 22.38(q); 18.85(q); 146: 169.70(s); 166.37(s); 136.13(s); 126.72(t); 99.39(s); 99.22(s); 79.38(s); 71.10(d); 66.32(d); 64.95(d); 61.92(t); 54.65(d); 37.81(t); 29.27(q); 27.69(q); 22.92(q); 19.68(q). Anal. calc. for $C_{19}H_{31}NO_8$ (401.47): C 56.84, H 7.78, N 3.49; found: C 56.57, H 7.56, N 3.33.

tert-Butyl 5-Acetamido-7,9-O-benzylidene-2,3,5-trideoxy-2-methylidene-D-glycero-D-galacto-nononate (147) and tert-Butyl 5-Acetamido-7,9-O-benzylidene-2,3,5-trideoxy-2-methylidene-D-glycero-D-talo-nononate (148). To an ice cold soln. of 143/144 (3.0 g, 6.67 mmol) in dioxane-H₂O 4:1 (200 ml) was added a soln. of AcOH (0.80 g, 13.34 mmol) in dioxane/H₂O 4:1 (10 ml). To this mixture 150 ml of a 0.13 molar soln. of NaBH₄⁴⁷ in dioxane-H₂O 4:1 were added dropwise during 12 h (= 1 drop per 8 seconds). According to TLC (CHCl₃/MeOH 9:1), 143/144 had disappeared.

The mixture was diluted with AcOEt (200 ml), excess NaBH₄ was destroyed with phosphate buffer (100 ml, pH 6.6). Extractive workup (half sat. aq. NaCl, brine) gave a 94:6 mixture of 147/148 (3.015 g) (HPLC; Zorbax-Sil, CH₂Cl₂/MeOH 97:3, 254 nm), which were separated by MPLC (500 g SiO₂, CH₂Cl₂/MeOH 95:5) affording 147 (2.604 g, 86.5%) and 148 (0.166 g, 5.5%) as colourless foams.

Data of 147. $[\alpha]_D^{25} = -37.3^\circ$ (c = 1.1, CHCl₃). IR: 3430m, 3005m, 2985m, 2935w, 2870w, 1703m, 1676s, 1630m, 1508m, 1370s, 1152s, 1086s, 1075s, 1030m, 1016 (sh). ¹H-NMR (400 MHz, (D₆)DMSO): 7.58-7.29 (m, NH and 5 arom. H); 6.00 (d, J=1.2, 1 olef. H); 5.60 (d, J=1.2, 1 olef. H); 5.35 (s, ArCH); 5.06 (d, J=5.8, OH); 4.47 (d, J=6.7, OH); 4.43 (d, J=7.9, OH); 4.15 (dd, J=10.5, 5.0, H-C(9)); 4.08-3.99 (m, H-C(4), H-C(5)); 3.85 (d (br.), J=10.0, H-C(6)); 3.74 (ddd, J=10.0, 9.0, 5.0, H-C(8)); 3.49 (dd, J=10.5, 10.0, H-C(9)); 4.43 (d (br.), J=9.0, H-C(7)); 2.23 (d, J=6.0, H₂-C(3)); 1.90 (s, CH₃); 1.42 (s, t-Bu). ¹³C-NMR (50 MHz): 170.90(s); 167.50(s); 138.58(s); 137.50(s); 128.80(d); 128.10(d); 127.30(t); 126.12(d); 100.67(d); 81.38(s); 80.46(d); 71.08(t); 69.59(d); 69.38(d); 61.12(d); 53.51(d); 37.44(t); 27.99(q); 23.18(q). Anal. calc. for C₂₃H₃₃NO₈ (451.53): C 61.18, H 7.37, N 3.10; found: C 61.41, H 7.25, N 3.30.

⁴⁷) Addition of solid NaBH₄ to the mixture diminished the diastereoselectivity of the reduction.

Data of 148. $[\alpha]_D^{25} = -18.1^\circ$ ($c = 1.1$, CHCl_3). IR: 3420s (br.), 3004m, 2985m, 2935m, 2870m, 1704 (sh), 1670s, 1632m, 1520m, 1370s, 1150s, 1086s, 1075s, 1015m. $^1\text{H-NMR}$ (400 MHz, $(\text{D}_6)\text{DMSO}$): 7.55-7.30 (m, NH, 5 arom. H); 6.00 (d, $J=2.0$, 1 olef. H); 5.58 (d, $J=2.0$, 1 olef. H); 5.41 (s, ArCH); 5.15 (d, $J=5.5$, OH); 4.72 (d, $J=7.8$, OH); 4.63 (d, $J=6.5$, OH); 4.15 (dd, $J=10.5$, 5.5, H-C(9)); 4.12 (dd, $J=9.0$, 6.0, H-C(5), after H/D exchange); 3.96 (d (br.), $J=9.0$, H-C(6), after H/D exchange); 3.82-3.74 (m, H-C(4), H-C(8)); 3.61 (d (br.), $J=10.0$, H-C(7)); 3.51 (dd, $J=10.5$, 10.5, H-C(9)); 2.50 (d (br.), $J=15.0$, H-C(3)); 2.20 (dd, $J=15.0$, 10.0, H-C(3)); 1.88 (s, CH_3); 1.40 (s, t-Bu). $^{13}\text{C-NMR}$ (50 MHz): 171.71(s); 167.09(s); 138.30(s); 137.55(s); 129.11(d); 128.28(d); 127.39(t); 126.08(d); 100.89(d); 81.98(d); 81.07(s); 71.64(d); 71.16(t); 67.46(d); 60.53(d); 56.78(d); 37.73(t); 27.96(q); 23.38(q). Anal. calc. for $\text{C}_{23}\text{H}_{33}\text{NO}_8$ (451.53): C 61.18, H 7.37, N 3.10; found: C 61.40, H 7.30, N 3.15.

tert-Butyl 5-Acetamido-2,3,5-trideoxy-7,9-O-isopropylidene-2-methylidene-D-glycero-D-galacto-nononate (149) and tert-Butyl 5-Acetamido-2,3,5-trideoxy-7,9-O-isopropylidene-2-methylidene-D-glycero-D-talo-nononate (150). Similarly to 143/144, 145/146 (1.50 g, 3.74 mmol) were reduced with $\text{NaBH}_4/\text{AcOH}$. After 10 h, TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1) indicated the disappearance of 145 and 146. The mixture was diluted with AcOEt (150 ml). Extractive workup with half sat. aq. NaCl (100 ml) and brine (100 ml) and FC (130 g SiO_2 , $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 19:1) gave a 92:8 mixture of 149 and 150 (1.25 g, 83%), (Anal. HPLC (Zorbax-Sil, CH_2Cl_2 -MeOH 193:7, λ_{obs} 228 nm) $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ showed only signals of 149. $^1\text{H-NMR}$ (200 MHz): 6.54 (d, $J=9.7$, NH); 6.19 (d, $J=1.5$, 1 H); 5.67 (s(br.), 1 H); 4.45-3.58 (m, 9 H, 2 H exchanged by addn. of D_2O); 3.32-3.18 (m, OH); 2.52-2.38 (m, $\text{H}_2\text{-C}(3)$); 2.04 (s, CH_3); 1.49 (s, t-Bu); 1.45 (s, CH_3); 1.39 (s, CH_3). $^{13}\text{C-NMR}$ (50 MHz): 170.47(s); 167.41(s); 138.65(s); 127.20(t); 99.01(s); 81.27(s); 72.55(d); 69.27(d); 64.61(t); 62.26(d); 53.30(d); 37.36(t); 28.45(q); 28.01(q); 23.17(q); 19.13(q). Anal. calc. for $\text{C}_{19}\text{H}_{33}\text{NO}_8$ (403.48): C 56.56, H 8.24, N 3.47; found: C 56.28, H 8.12, N 3.69.

Reduction of 143/144 and 145/146 in the absence of AcOH. An ice cold soln. of 143/144 (30 mg, 0.067 mmol) in dioxane-H₂O 4:1 (1.5 ml) was treated with NaBH₄ (6 x 1 mg, 2 1/2 h). Extractive workup (as detailed above) gave a 15:85 mixture of 147 and 148 (29 mg, 96%) as indicated by HPLC. Similarly, treatment of 145/146 (22 mg, 0.055 mmol) with NaBH₄ gave after extractive workup a 15:85 mixture of 149 and 150 (16 mg, 73%) as determined by ¹H-NMR. ¹H-NMR (200 MHz) of 150: 6.73 (d, J=8.3, NH); 6.15 (d, J=1.6, 1 H olef.); 5.66 (d, J=1.0, 1 H olef.); 4.34-3.54 (m, 10 H); 2.68 (dd, J=14.0, 1.0, H-C(3)); 2.35 (dd, J=14.0, 9.0, H-C(3)); 2.02 (s, CH₃); 1.55-1.36 (t-Bu, 2 x CH₃).

Reduction of 143/144 with NaBH₄ in EtOH. To an ice cold soln. of 143/144 (100 mg, 0.223 mmol) in EtOH (95%) (2 ml) was added NaBH₄ (50 mg). After 15 min. TLC indicated the disappearance of 143/144. Extractive workup with AcOEt and halfsat. aq. NaCl soln. gave a 15:85 mixture of 147 and 148 (100 mg, >95%).

Reduction of 143/144 with Zn(BH₄)₂ in THF. An ice cold soln. of 143/144 (100 mg, 0.223 mmol) in THF (2 ml) was treated with a 0.15 molar soln. of Zn(BH₄)₂ [191] in THF (2 ml). After 10 h, TLC indicated the disappearance of 143/144. Excess of Zn(BH₄)₂ was destroyed by adding phosphate buffer pH 6.6 (5 ml). Extractive workup (AcOEt) gave a 15:85 mixture of 147 and 148 (96 mg, 95%).

tert-Butyl 5-Acetamido-4,6,8-tri-O-acetyl-2,3,5-trideoxy-7,9-O-isopropylidene-2-methylidene-D-glycero-D-galacto-nononate (151) and tert-Butyl 5-Acetamido-4,6,8-tri-O-acetyl-2,3,5-trideoxy-7,9-O-isopropylidene-2-methylidene-D-glycero-D-talo-nononate (152). A soln. 149/150 (300 mg, 0.74 mmol) in Et₂O (3 ml) was acetylated at 0° with Ac₂O (1 ml) and pyridine (2 ml). After 3 h, TLC (CH₂Cl₂/MeOH 19:1) indicated the disappearance of the starting material. The mixture was concentrated and dried by evaporation with 2 x 10 ml toluene. The isomeres were separated by MPLC (100 g SiO₂, CH₂Cl₂/MeOH 40:1) yielding first 152 (220 mg, 56%) as a colourless foam and then 151 (133 mg, 34%) as colourless crystals.

Data of 151. M.p. 104-106°; $[\alpha]_D^{25} = -52.9^\circ$ (c = 1.2, CHCl₃). IR: 3435m, 2985m, 2935w, 2880w, 1740s, 1702(sh), 1687s, 1630w, 1502m, 1370s, 1316w, 1220s(br.), 1150s, 1068m, 1045s, 1030s, 850m. ¹H-NMR (200 MHz): 6.08 (d, J=2.0, 1 olef. H); 5.85 (d, J=10.0, NH); 5.50 (s(br.), 1 olef. H); 5.29 (ddd, J=9.5, 4.0, 3.0, H-C(4)); 5.01 (dd, J=8.5, 2.0, H-C(6)); 4.78-4.56 (m, H-C(5) and H-C(8)); 4.13 (dd, J=10.0, 2.0, H-C(7)); 3.99 (dd, J=11.5, 5.5, H-C(9)); 3.63 (dd, J=11.5, 8.5, H-C(9)); 2.61 (dd, J=14.0, 3.0, H-C(3)); 2.23 (dd, J=14.0, 9.5, H-C(3)); 2.12-1.90 (4 x CH₃); 1.67-1.37 (5 x CH₃). ¹³C-NMR (25.2 MHz): 170.06(s); 169.68(s); 169.64(s); 169.30(s); 165.27(s); 137.35(s); 126.81(t); 99.88(s); 80.79(s); 70.25(d); 69.42(d); 67.87(d); 64.64(d); 61.78(t); 50.39(d); 35.32(t); 28.02(q); 27.71(q); 23.42(q); 20.82(q); 20.66(q); 19.30(q). MS(EI): 514 (M⁺ - 15, 3); 458(7); 414(2); 398(5); 356(8); 316(8); 296(4); 270(6); 228(36); 198(15); 186(16); 168(50); 115(51); 57(23); 43(100). Anal. calc. for C₂₅H₃₉NO₁₁ (529.60): C 56.70, H 7.42, N 2.65; found: C 56.44, H 7.55, N 2.49.

Data of 152. $[\alpha]_D^{25} = +20.3^\circ$ (c = 1.2, CHCl₃). IR: 3425m, 2995m, 2985m, 2935w, 2880w, 1743s, 1705s, 1680s, 1632w, 1510m, 1370s, 1343w, 1310w, 1238s, 1153s, 1052s. ¹H-NMR (200 MHz): 6.57 (d, J=10.0, NH); 6.08 (d, J=2.0, 1 olef. H); 5.56 (s, 1 olef. H); 5.24-5.06 (m, H-C(4) and H-C(6)); 4.79 (ddd, J=10.0, 8.5, 5.5, H-C(8)); 4.60 (ddd, J=10.0, 10.0, 5.5, H-C(5)); 4.17 (dd, J=10.0, 2.0, H-C(7)); 3.99 (dd, J=11.5, 5.5, H-C(9)); 3.65 (dd, J=11.5, 8.5, H-C(9)); 2.89 (dd, J=14.0, 1.5, H-C(3)); 2.26 (dd, J=14.0, 10.0, H-C(3)); 2.16-1.96 (4 x CH₃); 1.66-1.42 (5 x CH₃). ¹³C-NMR (25.2 MHz): 169.91(s); 169.83(s); 169.45(s); 169.15(s); 164.97(s); 137.16(s); 126.55(t); 99.58(s); 80.30(s); 71.07(d); 70.71(d); 66.39(d); 63.62(d); 61.44(t); 51.78(d); 35.53(t); 27.80(q); 23.25(q); 20.63(q); 20.40(q); 19.34(q). EI-MS: 514 (1, M⁺ - 15), 471(3). Anal. calc. for C₂₅H₃₉NO₁₁ (529.60): C 56.70, H 7.42, N 2.65; found: C 56.44, H 7.27, N 2.52.

tert-Butyl 5-Acetamido-7,9-O-benzylidene-3,5-dideoxy-D-glycero-D-galacto-2-nonulosonate (153). At -78° , a soln. of 147 (100 mg, 0.221 mmol) in CH_2Cl_2 (7 ml) was ozonized until the soln. turned blue.⁴⁸ It was purged with N_2 (5 min), treated with a soln. of PPh_3 (87 mg, 0.332 mmol) in CH_2Cl_2 (1 ml) and warmed to r.t.. Evaporation of the solvent and flash chromatography of the residue (10 g SiO_2 , $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 93:7) gave 153 (89 mg, 89%) as a colourless foam. An analytical sample was obtained by crystallization from anh. EtOH/hexane. M.p. $167-168^{\circ}$ (dec.). $[\alpha]_{\text{D}}^{25} = -62.8^{\circ}$ (20 h, $c = 1.0$, CHCl_3). IR (KBr): 3400s (br.), 2975m, 2905w, 2855w, 1740s, 1632s, 1567m, 1384s, 1370s, 1328m, 1142s, 1124s, 1088s, 1036s, 1026s. $^1\text{H-NMR}$ (400 MHz, $(\text{D}_6)\text{DMSO}$): 7.65 (d, $J=8.6$, NH); 7.56-7.30 (m, 5 arom. H); 6.15 (d, $J=2.0$, OH); 5.31 (s, ArCH); 4.94 (d, $J=5.5$, OH); 4.71 (d, $J=6.0$, OH); 4.16 (dd, $J=10.4$, 5.3, H-C(9)); 4.00 (dd, $J=9.1$, 0.5, H-C(6)); 3.93-3.83 (m, H-C(4), H-C(5), H-C(8)); 3.48 (dd, $J=10.0$, 0.5, H-C(7)); 3.46 (dd, $J=10.4$, 10.0, H-C(9)); 1.97 (dd, $J=13.0$, 4.6, H-C(3)); 1.85 (s, CH_3); 1.58 (dd, $J=13.0$, 11.0, H-C(3)); 1.41 (s, t-Bu); addn. of D_2O : 3.81 (ddd, $J=9.5$, 9.1, 8.6, H-C(5)); 3.75 (ddd, $J=11.0$, 9.5, 4.6, H-C(4)); 3.70 (ddd, $J=10.0$, 10.0, 5.3, H-C(8)). $^{13}\text{C-NMR}$ (50 MHz, CD_3OD): 173.67(s); 170.22(s); 139.46(s); 129.42(d); 128.73(d); 127.45(d); 102.17(d); 96.51(s); 83.29(s); 80.64(d); 72.11(t); 70.01(d); 68.76(d); 61.41(d); 52.84(d); 40.16(t); 28.06(q); 22.96(q). Anal. calc. for $\text{C}_{22}\text{H}_{31}\text{NO}_9$ (453.50): C 58.27, H 6.89, N 3.09; found: C 57.99, H 7.11, N 2.95.

tert-Butyl 5-Acetamido-7,9-O-benzylidene-3,5-dideoxy-D-glycero-D-talo-2-nonulosonate (154). A sample of 148 (250 mg, 0.554 mmol) was ozonized as described for 12. The crude product was purified by FC (25 g SiO_2). $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (97:3) eluted PPh_3 and Ph_3PO ; $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95:5 gave 154 (225 mg, 90%) as a colourless foam. Crystallization from anh. MeOH (0.5 ml) and Et_2O yielded 154 (195 mg). M.p. $164-165^{\circ}$ (dec.). $[\alpha]_{\text{D}}^{25} = -80.9^{\circ}$ ($c = 1.0$, CHCl_3). IR(KBr): 3500s, 3415s, 2985w, 1738s, 1640s, 1536m, 1385m, 1370m, 1320m, 1310m, 1150s, 1095s, 1080s, 1060m, 1020m, 850w, 770m.

⁴⁸) Continuing the ozonolysis led to by-products, which are likely formed by oxidation of the benzylidene group [280].

$^1\text{H-NMR}$ (400 MHz, $(\text{D}_6)\text{DMSO}$): 7.70 (d, $J=9.4$, NH); 7.50-7.28 (m, 5 H arom.); 6.48 (s, OH-C(2)); 5.34 (s, ArCH); 5.10 (d, $J=6.0$, OH-C(4)); 5.05 (d, $J=5.6$, OH-C(8)); 4.36 (dd, $J=10.5$, 1.0, H-C(6)); 4.16 (dd, $J=10.5$, 5.0, H-C(9)); 4.11 (ddd, $J=10.5$, 9.4, 3.0, H-C(5)); 3.93 (dd (br.), $J=6.0$, 3.0, H-C(4), addn. of $\text{D}_2\text{O} \rightarrow$ broad d); 3.78 (dddd, $J=10.5$, 9.5, 5.6, 5.0; H-C(8), addn. of $\text{D}_2\text{O} \rightarrow$ ddd); 3.57 (dd, $J=9.5$, 1.0, H-C(7)); 3.48 (dd, $J=10.5$, 10.5, H-C(9)); 1.98-1.90 (m, $\text{H}_2\text{-C}(3)$); 1.87 (s, CH_3); 1.40 (s, t-Bu). $^1\text{H-NMR}$ (200 MHz, CD_3OD): $\beta\text{-D}/\alpha\text{-D} = 3:1$; $\beta\text{-D}$ -anomer: 7.60-7.26 (m, 5 arom. H); 5.44 (s, ArCH); 4.46 (dd, $J=10.5$, 1.8, H-C(6)); 4.33 (dd, $J=10.5$, 3.0, H-C(5)); 4.27 (dd, $J=10.5$, 5.5, H-C(9)); 4.10 (ddd, $J=3.4$, 3.0, 3.0, H-C(4)); 3.98 (ddd, $J=10.5$, 9.0, 5.5, H-C(8)); 3.64 (dd, $J=9.0$, 1.8, H-C(7)); 3.58 (dd, $J=10.5$, 10.5, H-C(9)); 2.17 (dd, $J=14.0$, 3.0, H-C(3)); 2.03 (dd, $J=14.0$, 3.4, H-C(3)); 2.01 (s, CH_3); 1.47 (s, t-Bu); $\alpha\text{-D}$ -anomer: 4.55 (dd, $J=10.5$, 1.8, H-C(6)); 2.60 (dd, $J=14.0$, 3.0, H-C(3)); 1.98 (s, CH_3); 1.76 (dd, $J=14.0$, 2.5, H-C(3)); 1.51 (s, t-Bu); (the other signals were overlapped by the signals of the $\beta\text{-D}$ -anomer). $^{13}\text{C-NMR}$ (50 MHz, CD_3OD): $\beta\text{-D}$ -anomer: 172.94(s); 169.62(s); 139.45(s); 129.52(d); 128.84(d); 127.41(d); 102.22(d); 96.67(s); 83.45(s), 80.96(d); 72.18(t); 67.88(d); 65.98(d); 61.00(d); 48.15(d); 37.04(t); 28.05(q); 22.73(q); $\alpha\text{-D}$ -anomer: 172.94(s); 171.74(s); 139.45(s); 129.52(d); 128.84(d); 127.41(d); 102.22(d); 95.73(s); 83.21(s); 81.27(d); 72.26(t); 69.49(d); 67.30(d); 61.51(d); 41.67(t); 28.05(q); 22.73(q); Anal. calc. for $\text{C}_{22}\text{H}_{31}\text{NO}_9$ (453.50): C 58.27, H 6.89, N 3.09; found: C 58.02, H 6.99, N 2.90.

tert-Butyl 5-Acetamido-3,5-dideoxy-7,9-O-isopropylidene-D-glycero-D-galacto-2-nonulosonate (155) and tert-Butyl 5-Acetamido-3,5-dideoxy-7,9-O-isopropylidene-D-glycero-D-talo-2-nonulosonate (156). A mixture of 149/150 (1.012 g, 2.50 mmol) was ozonized as described for 147. The crude products were purified by flash chromatography (150 g SiO_2), $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 15:1 eluted 156 (70 mg, 7%) as a colourless foam and $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 12:1 155 (861 mg, 85%) as an oil, which solidified after a few hours.

Data of 155. $[\alpha]_{\text{D}}^{25} = -44.8^\circ$ ($c = 1.0$, MeOH). IR(KBr): 3410s(br.), 2985w, 2940w, 1734m, 1643s, 1553w, 1370s, 1315w, 1227w, 1150s, 1125s, 1070s, 1030m. $^1\text{H-NMR}$ (400 MHz, $(\text{D}_6)\text{DMSO}$): 7.58 (d, $J=8.3$, NH); 6.11

(s, OH-C(2)); 4.78 (d, J=5.2, OH); 4.66 (d, J=5.5, OH); 4.06 (dd, J=9.0, 1.5, H-C(6)); 3.85-3.64 (m, H-C(4), H-C(5), H-C(8), H-C(9)); addn. of D₂O: 3.82 ppm, ddd, J=11.0, 9.8, 4.5, H-C(4)); 3.62 (dd, J=9.2, 1.5, H-C(7)); 3.48 (dd, J=10.0, 9.5, H-C(9)); 2.03 (dd, J=12.6, 4.5, H-C(3)); 1.83 (s, CH₃); 1.71 (dd, J=12.6, 11.0, H-C(3)); 1.44 (s, t-Bu); 1.29 (s, CH₃); 1.25 (s, CH₃). ¹³C-NMR (50 MHz, CD₃OD): 173.17(s); 170.24(s); 100.20(s); 96.51(s); 83.26(s); 72.63(d); 70.02(d); 69.06(d); 65.66(t); 62.78(d); 52.72(d); 40.22(t); 28.62(q); 28.05(q); 22.98(q); 19.06(q). Anal. calc. for C₁₈H₃₁NO₉ (405.46): C 53.32, H 7.71, N 3.46; found: C 53.06, H 7.90, N 3.54.

Data of 156. $[\alpha]_D^{25} = -63.7^\circ$ (c = 1.0, CHCl₃). IR: 3420m(br.), 2985s, 2930m, 2870w, 1730s, 1670s, 1505m, 1370s, 1306m, 1136s, 1090s, 1064s, 1024m. ¹H-NMR (400 MHz, (D₆)DMSO): 7.63 (d, J=9.2, NH); 6.40 (s, OH-C(2)); 5.01 (d, J=6.2, OH); 4.84 (d, J=5.0, OH); 4.28 (dd, J=10.5, 1.2, H-C(6)); 3.95 (ddd, J=10.5, 9.2, 3.0, H-C(5)); 3.87 (ddd, J=5.8, 5.8, 3.0, H-C(4)); 3.71 (dd, J=9.8, 4.0, H-C(9)); 3.69-3.59 (m, H-C(8)); 3.61 (dd, J=9.5, 1.2, H-C(7)); 3.43 (dd, J=9.8, 8.2, H-C(9)); 1.92 (d, J=3.0, H₂-C(3)); 1.80 (s, CH₃); 1.42 (s, t-Bu); 1.23 (s, CH₃); 1.19 (s, CH₃). CI-MS: 406 ([M + 1]⁺), 388 ([M + 1]⁺-18), 350 ([M + 1]⁺-56), 332 ([M + 1]⁺-74). Anal. calc. for C₁₈H₃₁NO₉ (405.46): C 53.32, H 7.71, N 3.46; found: C 53.51, H 7.79, N 3.26.

tert-Butyl 5-Acetamido-3,5-dideoxy-D-glycero-D-galacto-2-nonulosonate (157)

A) From 153. To a suspension of 10% Pd/C (150 mg) in MeOH/H₂O 4:1 (20 ml) and AcOH (0.6 ml) was added a soln. of 153 (610 mg, 1.35 mmol) in MeOH (5 ml). The mixture was hydrogenated (24 h) until 153 had disappeared (TLC, CHCl₃/MeOH 9:1). The mixture was filtered through celite, washed with MeOH and the filtrate was evaporated. A soln. of the residue in anh. MeOH (10 ml), was filtered through cotton and concentrated. For crystallization, a stirred soln. of the residual colourless oil dissolved in anh. MeOH (5 ml) was diluted with AcOEt (7 ml) and then with Et₂O until persistence of a slight turbidity. Additional Et₂O (5 ml) was added after 16 h and the mixture was kept in the refrigerator (1 d). The crystals were dried (P₂O₅, 10⁻⁵ mbar, 2 d) affording 157 (422 mg, 85%). The mother liquors were chromatographed (AcOEt/MeOH/H₂O 170:27:3) on a preconditio-

ned column (20 g SiO₂, AcOEt/MeOH 98:2) to give additional 157 (45 mg, 9%). M.p. 178-179° (dec.). $[\alpha]_D^{25} = -24.8^\circ$ (c = 1.0, H₂O). IR(KBr): 3460s, 2980m, 2940m, 1726s, 1635s, 1555s, 1370s, 1312s, 1135s, 1030s. ¹H-NMR (400 MHz, D₂O): 4.10-4.01 (m, H-C(4), H-C(6)); 3.91 (dd, J=10.4, 10.4, H-C(5)); 3.86 (dd, J=12.0, 2.4, H-C(9)); 3.76 (ddd, J=9.4, 6.4, 2.4, H-C(8)); 3.63 (dd, J=12.0, 6.4, H-C(9)); 3.57 (d (br.), J=9.4, H-C(7)); 2.69 (dd, J=12.0, 4.8, 0.1 H-C(3)); 2.31 (dd, J=13.0, 4.8, 0.9 H-C(3)); 2.06 (s, 0.9 CH₃); 2.05 (s, 0.1 CH₃); 1.87 (dd, J=13.0, 12.0, H-C(3)); 1.53 (s, 0.1 t-Bu); 1.52 (s, 0.9 t-Bu). ¹³C-NMR (100.6 MHz, CD₃OD): β-D-anomer: 175.05(s); 170.84(s); 96.71(s); 83.85(s); 72.26(d); 72.11(d); 70.47(d); 68.01(d); 64.91(t); 54.43(d); 40.83(t); 28.09(q); 22.68(q); α-D-anomer: 175.32(s); 170.84(s); 97.38(s); 84.59(s); 74.90(d); 72.71(d); 70.29(d); 68.90(d); 64.81(t); 54.05(d); 42.47(t); 28.09(q); 22.68(q). CI-MS: 366 ([M + 1]⁺), 310, 292, 274. Anal. calc. for C₂₅H₂₇NO₉ (365.39): C 49.31, H 7.45, N 3.83; found: C 49.26, H 7.50, N 3.99.

B) From 155. An ice cold soln. of 155 (160 mg, 0.395 mmol) in CH₂Cl₂/MeOH 1:1 (5 ml) was treated with CF₃COOH (100 μl). The mixture was slowly warmed to r.t. (melting ice bath). After 6 h, TLC indicated the disappearance of 155. The solvent was removed, the residue was dissolved in H₂O (7 ml) and extracted with CH₂Cl₂. The aq. layer was freeze dried giving crude 157 (148 mg, quant.). Crystallization from anh. MeOH (1.5 ml), AcOEt (1.5 ml) and Et₂O (cf. 153→157) yielded 157 (124 mg, 86%). M.p. 176-177°. $[\alpha]_D^{25} = -25.0^\circ$ (c = 1.0, H₂O).

5-Acetamido-3,5-dideoxy-D-glycero-D-galacto-2-nonulosonic Acid (=N-Acetylneuraminic Acid; Neu5Ac, 2). A solution of 157 (300 mg, 0.821 mmol) in MeOH/H₂O 3:2 (15 ml) was stirred with K₂CO₃ (150 mg) at r.t. (4 h) until TLC (AcOEt/MeOH/0.1 n HCl 2:2:1) indicated the disappearance of 157. MeOH was evaporated, H₂O (5 ml) was added to the residue, the soln. was acidified to pH 4 (Dowex 50 W 4 (H⁺)) and stirred at r.t. for 30 minutes. The resin was filtered off, resuspended in H₂O (3 ml) and stirred for another 30 minutes. The filtrate and washings were combined and freeze dried to give crude 2 (254 mg, 100%). Crystallization (0.4 ml H₂O, 6.0 ml AcOH, 3 d, 4°) [6],[72] gave after drying (P₂O₅, KOH, 10⁻⁵ mbar) 1 (130 mg, 51%). M.p. 180-182° (dec.) (Lit.[72]: 181-183°). $[\alpha]_D^{20} = -33.0^\circ$ (c = 1.0, H₂O); (Lit.[72]: -32.1° (c = 1.3, H₂O)).

Methyl 5-Acetamido-3,5-dideoxy-D-glycero-D-galacto-2-nonulosonate (160)

A sample of 157 (100 mg, 0.274 mmol) was treated with K_2CO_3 as described for 2. A soln. of the crude Neu5Ac (2, 90 mg) in anh. MeOH (9 ml), was stirred with CF_3COOH (40 μ l) at r.t. for 24 h. The solvent was removed and the residual oil was dried at 10^{-2} mbar for 2 h. A soln. of the residue in anh. MeOH (0.7 ml) and AcOEt (0.7 ml) was treated with Et_2O and kept in the refrigerator (5 d). The crystals were dried (P_2O_5 , 10^{-5} mbar) yielding 160 (52 mg, 59%). M.p. 176-178° (Lit.[194]: 179-180°); $[\alpha]_D^{25} = -28.6^\circ$ (c = 1.1, H_2O), (Lit.[194]: -28° (c = 1.0, H_2O)).

tert-Butyl 5-Acetamido-3,5-dideoxy-D-glycero-D-talo-nonulosonate (158).

A) From 154. A sample of 154 (130 mg, 0.287 mmol) was hydrogenated as described for 153→157 (TLC: $CH_2Cl_2/EtOH$ 9:1). The crude 158 (104 mg) was crystallized from MeOH (1 ml), AcOEt (1.5 ml) and Et_2O (10 ml) affording pure 158 (73 mg, 70%). The mother liquors were chromatographed (AcOEt/MeOH/ H_2O 170:27:3) on a preconditioned column (15 g SiO_2 , AcOEt) to give 158 (27 mg, 25%). M.p. 157-158°. $[\alpha]_D^{25} = -76.0^\circ$ (c = 1.0, H_2O). IR(KBr): 3500(sh), 3360s, 2980w, 2940w, 1733s, 1668s, 1520m, 1430m, 1370m, 1309m, 1136s, 1089s, 1050m, 1027m, 1012m, 918m, 843m. 1H -NMR (400 MHz, D_2O): 4.38 (d (br.), J=11.0, 0.7 H-C(6)); 4.34 (d (br.), J=11.0, 0.3 H-C(6)); 4.22-4.17 (m, H-C(4)); 4.14 (dd, J=11.0, 3.0, 0.7 H-C(5)); 4.08 (dd, J=11.0, 2.5, 0.3 H-C(5)); 3.97-3.78 (m, 2 H; including a dd at 3.87, J=11.8 and 2.6 for 0.7 H-C(9) and a ddd at 3.81, J=9.0, 6.3, 2.6, for 0.7 H-C(8)); 3.71-3.62 (m, 1 H; including a dd at 3.65, J=11.8 and 6.3 for 0.7 H-C(9)); 3.61 (d (br.), J=9.0, 0.7 H-C(7)); 3.57 (d (br.), J=9.0, 0.3 H-C(7)); 2.68 (dd, J=14.0, 3.6, 0.3 H-C(3)); 2.17 (d, J=3.0, 0.7 H-C(3)); 2.06 (s, 0.7 CH_3); 2.04 (s, 0.3 CH_3); 1.89 (dd, J=14.0, 2.7, 0.3 H-C(3)); 1.52 (s, t-Bu). ^{13}C -NMR (25 MHz, CD_3OD): β -D-anomer: 172.57(s); 170.13(s); 95.36(s); 83.70(s); 71.12(d); 68.59(d); 66.86(d); 66.19(d); 62.91(t); 47.98(d); 36.44(t); 26.70(q); 21.34(q); α -D-anomer: 172.86(s); 170.46(s); 94.12(s); 82.34(s); 71.68(d); 70.24(d); 69.03(d); 65.75(d); 63.35(t); 48.32(d); 40.28(t); 26.70(q); 21.34(q). CI-MS: 366 ($[M + 1]^+$), 348, 310, 292, 274. Anal. calc. for $C_{15}H_{27}NO_9$ (365.39): C 49.31, H 7.45, N 3.83; found: C 49.07, H 7.70, N 4.01.

B) From 156. Similarly to 155, deprotection of 156 (280 mg, 0.690 mmol) and crystallization of the product, gave 158 (164 mg, 65%). M.p. 156-158° (dec.). $[\alpha]_D^{25} = -76.4^\circ$ (c = 1.0, H₂O).

Sodium 5-Acetamido-3,5-dideoxy-D-glycero-D-talo-2-nonulosonate (Sodium N-Acetyl-4-epineuraminatate; 159). Similarly to 157, 158 (350 mg, 0.958 mmol) was saponified (TLC: CHCl₃/MeOH 3:1 and n-Propanol/MeOH/0.1 n HCl 5:3:2). Crude 4-epi-Neu5Ac (295 mg) was purified by anion exchange chromatography (35 ml Dowex 1X8 (HCOO⁻), elution by 0-0.4 N aq. HCOOH). Fractions containing 4-epi-Neu5Ac (159, R=H) were collected, concentrated and finally freeze-dried. The red-brown residual oil was decolourized by activated charcoal.⁴⁹⁾ The residual slightly yellow oil was transformed to the sodium salt by passing it through a cation exchanger (10 ml Dowex 50W X 4 (Na⁺), H₂O) to give after freeze-drying 159 (146 mg, 46%, 5d 10⁻⁵ mbar) as a colourless solid. $[\alpha]_D^{25} = -71.4^\circ$ (c = 1.0, H₂O). ¹H-NMR (400 MHz, D₂O): α-D / β-D = 1:9; β-D-anomer: 4.30 (dd, J=10.8, 1.2, H-C(6)); 4.19 (ddd, J=3.3, 3.2, 3.0, H-C(4)); 4.14 (dd, J=10.8, 3.0, H-C(5)); 3.87 (dd, J=11.5, 2.8, H-C(9)); 3.86-3.80 (m, H-C(8)); 3.64 (dd, J=11.5, 6.1, H-C(9)); 3.55 (dd, J=8.9, 1.2, H-C(7)); 2.12 (dd, J=14.7, 3.3, H-C(3)); 2.08 (dd, J=14.7, 3.2, H-C(3)); 2.05 (s, CH₃); α-D-anomer: 2.49 (dd, J=14.7, 3.2, H-C(3)); 2.04 (s, CH₃); 1.99 (dd, J=14.7, 3.7, H-C(3)); the signals for H-C(4) - H₂-C(9) are covered by the signals of the β-D-anomer. ¹³C-NMR (50 MHz, D₂O): β-D-anomer: 177.10(s); 174.33(s); 96.54(s); 70.52(d, C(8)); 69.05(d, C(7)); 66.62(d, C(4)); 66.07(d, C(6)); 63.63(t); 48.11(d); 36.76(t); 22.34(q); α-D-anomer: 176.52(s); 174.54(s); 95.96(s); 71.22(d); 70.03(d); 63.39(t); 48.67(d); 38.41(t); the signals for C(4) and C(6) were determined by selective ¹H, ¹³C-decoupling experiments. FAB-MS: 332 ([M + 1]⁺). Anal. calc. for C₁₁H₁₈NNaO₉ (331.28): C 39.88, H 5.48, N 4.24; found: C 39.59, H 5.71, N 3.99.

⁴⁹⁾ Activated by treating it with 0.5 N HCOOH (2 h) and washing with H₂O (bidest).

Methyl 5-Acetamido-3,5-dideoxy-D-glycero-D-talo-2-nonulosonate (161)

A suspension of 158 (120 mg, 0.328 mmol) in chlorobutane (1.5 ml) was treated with CF_3COOH (1 ml) and stirred at r.t. (6 h). TLC ($\text{CHCl}_3/\text{MeOH}$ 3:1) indicated then the disappearance of 158. The soln. was concentrated and twice co-evaporated with anh. MeOH (2 x 3 ml). The residual oil was redissolved in anh. MeOH (3 ml), treated with CF_3COOH (100 μl) and stirred at r.t. (2 d). The soln. was concentrated and chromatographed (20 g SiO_2 , $\text{CHCl}_3/\text{MeOH}$ 9:1) on a preconditioned column (24 h). Fractions containing 161 were combined, concentrated and extracted with H_2O . The H_2O layer was freeze-dried to give 161 (100 mg, 94%) as a microcrystalline solid. An analytical sample was obtained by crystallization from anh. MeOH (1 ml), AcOEt (1 ml) and Et_2O (12 ml). M.p. 166-168° (dec.). $[\alpha]_{\text{D}}^{20} = -67^\circ$ (c = 1.0, H_2O). IR (KBr): 3380 s(br.), 1740m, 1655m, 1545m. $^1\text{H-NMR}$ (400 MHz, D_2O): $\alpha\text{-D}/\beta\text{-D} = 1:3$. $\beta\text{-D}$ -anomer: 4.39 (dd, $J=10.6, 1.4$, H-C(6)); 4.20 (ddd, $J=3.0, 3.0, 3.0$, H-C(4)); 4.15 (dd, $J=10.6, 3.0$, H-C(5)); 3.85 (dd, $J=11.6, 2.4$, H-C(9)); 3.83 (s, CH_3); 3.79 (dd, $J=9.0, 6.2, 2.4$, H-C(8)); 3.63 (dd, $J=11.6, 6.2$, H-C(9)); 3.58 (dd, $J=9.0, 1.4$, H-C(7)); 2.19 (d, $J=3.0$, H-C(3)); 2.05 (s, CH_3); $\alpha\text{-D}$ -anomer: 4.33 (dd, $J=11.0, 1.8$, H-C(6)); 4.09 (dd, $J=11.0, 3.0$, H-C(5)); 3.82 (s, CH_3); 2.69 (dd, $J=14.4, 3.6$, H-C(3)); 2.03 (s, CH_3); 1.96 (dd, $J=14.4, 2.6$, H-C(3)); the signals for H-C(4), H-C(7)-H-C(9) were overlapped by the signals of the $\beta\text{-D}$ -anomer. $^{13}\text{C-NMR}$ (50 MHz, D_2O): $\beta\text{-D}$ -anomer: 174.58(s); 171.96(s); 95.66(s); 70.52(d); 68.90(d); 66.57(d); 66.06(d); 63.69(t); 54.03(q); 48.01(d); 36.57(t); 22.45(q); $\alpha\text{-D}$ -anomer: 174.77(s); 94.61(s); 71.67(d); 70.69(d); 69.01(d); 63.51(t); 53.58(q); 48.28(d); 39.81(t). FAB-MS: 324 ($[\text{M} + 1]^+$). Anal. calc. for $\text{C}_{12}\text{H}_{21}\text{NO}_9$ (323.31): C 44.58, H 6.55, N 4.33; found: C 44.34, H 6.69, N 4.14.

5. Synthesis of N-Acetyl-4-deoxyneuraminic Acid

tert-Butyl 5-Acetamido- 4,6,8-tri-O-acetyl- 7,9-O-benzylidene-2,3,5- tri-deoxy-2-methylidene-D-glycero-D-galacto-nononate (163) and tert-Butyl 5-Acetamido- 4,6,8-tri-O-acetyl-7,9-O-benzylidene-2,3,5-trideoxy-2-methylidene-D-glycero-D-talo-nononate (164). A soln. of 143 (1.50 g, 3.34 mmol) in THF (25 ml) was treated with NH_4Cl (180 mg, 3.51 mmol) and heated to 50° . NaBH_4 (120 mg) was added in one portion. The soln. changed to yellow. After 30 min, TLC ($\text{CH}_2\text{Cl}_2/\text{EtOH}$ 9:1) of the colourless soln. indicated the disappearance of 143. Excess NaBH_4 was destroyed with phosphate buffer pH 6.6 (15 ml). The mixture was diluted with AcOEt (100 ml) and extracted with H_2O and brine to give a mixture of 147/148 as a colourless foam (D-glycero-D-galacto/D-glycero-D-talo = 1 : 3 as indicated by HPLC (see above). An ice-cold soln. of 147/148 in pyridine (10 ml) and Ac_2O (5 ml) was stirred at 0° (5 h), then at r.t. (over night). The mixture was concentrated and dried by co-evaporation with 3 x 10 ml toluene. FC of the residue (50 g SiO_2 , $\text{AcOEt}/\text{hexane}$ 7:3) gave a mixture of 163 and 164 (1.61 g, 83%) and the mono-O-acetate 167 (240 mg). Acetylation of the mono-O-Acetate 167 ($\text{Py}/\text{Ac}_2\text{O}$) gave further 164 (266 mg, 14%). Anal. samples of 163 and 164 were obtained by FC ($\text{AcOEt}/\text{hexane}$ 1:1) and crystallization from ether/hexane.

Data of 163. M.p. $159-161^\circ$. $[\alpha]_{\text{D}}^{25} = -28.5^\circ$ ($c = 1.0$, CHCl_3). IR: 3435m, 3035w, 3005m, 2985m, 2935m, 2870w, 1745s(br.), 1686s, 1635w, 1507m, 1370s, 1314w, 1225s(br.), 1156s, 1088s, 1048s, 1030s. $^1\text{H-NMR}$ (200MHz): 7.69-7.32 (m, 5 arom. H); 6.10 (d, $J=1.0$, 1 olef. H); 5.89 (d, $J=10.0$, NH); 5.51 (s(br.), ArCH, 1 olef. H); 5.36 (ddd, $J=9.0$, 3.5, 3.5, H-C(4)); 5.05 (dd, $J=8.5$, 1.8, H-C(6)); 4.89-4.69 (m, H-C(5), H-C(8)); 4.42 (dd, $J=10.2$, 5.2, H-C(9)); 4.03 (dd, $J=10.0$, 1.8, H-C(7)); 3.60 (dd, $J=10.2$, 10.0, H-C(9)); 2.60 (dd, $J=14.0$, 3.5, H-C(3)); 2.42 (dd, $J=14.0$, 9.0, H-C(3)); 2.05 (s, $2\times \text{CH}_3$); 2.03 (s, CH_3); 1.97 (s, CH_3); 1.47 (s, t-Bu). $^{13}\text{C-NMR}$ (25.2 MHz): 169.97(s); 169.50(s); 169.48(s); 169.33(s); 165.17(s); 137.16(s); 136.82(s); 128.68(d); 127.97(d); 126.94(t); 125.89(d); 101.13(d); 80.79(s); 77.00(d); 70.08(d); 67.77(t); 67.47(d); 62.16(d); 49.90(d); 35.24(t); 27.91(q); 23.30(q); 20.95(q); 20.80(q); 20.63(q). EI-MS: cf. 164. Anal. calc. for $\text{C}_{29}\text{H}_{39}\text{NO}_{11}$ (577.63): C 60.30, H 6.81, N 2.42; found: C 60.02, H 6.90, 2.69.

Data of 164. M.p. 101-103°. $[\alpha]_D^{25} = +26.0^\circ$ (c = 1.0, CHCl₃). IR: 3430m, 3035w, 3005m, 2985m, 2940w, 2870w, 1746s(br.), 1682s, 1631w, 1510m, 1370s, 1310m, 1225s(br.), 1156s, 1090s, 1050s, 1028s, 1015s. ¹H-NMR (200 MHz): 7.62-7.36 (m, 5 arom. H); 6.53 (d, J=10.0, NH); 6.09 (d, J=1.5, 1 olef. H); 5.56 (s, ArCH); 5.54 (d, J=1.5, 1 olef. H); 5.25 (dd, J=5.5, 2.0, H-C(6)); 5.23 (ddd, J=9.5, 9.0, 3.0, H-C(4)); 4.95 (ddd, J=10.5, 10.0, 5.5, H-C(8)); 4.62 (ddd, J=10.0, 9.0, 5.5, H-C(5)); 4.38 (dd, J=10.5, 5.5, H-C(9)); 4.17 (dd, J=10.0, 2.0, H-C(7)); 3.63 (dd, J=10.5, 10.5, H-C(9)); 2.82 (dd, J=14.0, 3.0, H-C(3)); 2.30 (dd, J=14.0, 9.5, H-C(3)); 2.08 (s, CH₃); 2.05 (s, CH₃); 2.03 (s, CH₃); 1.83 (s, CH₃); 1.44 (s, t-Bu). ¹³C-NMR (25.2 MHz): 170.18(s); 170.02(s); 169.68(s); 169.19(s); 165.14(s); 137.01(s); 136.64(s); 129.41(d); 128.35(d); 127.18(t); 125.83(d); 101.46(d); 80.63(s); 78.48(d); 71.71(d); 67.92(t); 65.99(d); 61.38(d); 52.08(d); 35.56(t); 27.95(q); 23.24(q); 20.90(q); 20.60(q); 20.53(q). EI-MS: 577(0.3, M⁺); 522(0.5); 520(0.8); 517(0.4); 471(0.7); 464(2); 462(2); 415(1); 364(6); 356(4); 302(11); 258(33); 228(25); 186(11); 168(48); 115(44); 57(56); 43(100). Anal. calc. for C₂₉H₃₉NO₁₁ (577.63): C 60.30, H 6.81, N 2.42; found: 60.13, H 6.79, N 2.35.

tert-Butyl (E)-5-Acetamido- 6,8-di-O-acetyl-7,9-O-benzylidene-3,4,5-tri-deoxy-D-manno-non-3-en-2-ulosonate (165). A mixture of 163/164 (58 mg, 0.10 mmol) was ozonized and treated with (i-Pr)₂EtN as described for 162. FC (5 g SiO₂, AcOEt/hexane 1:1) gave 165 (47 mg, 91%)⁵⁰. IR: 3430m, 3030w, 2985m, 2930w, 2870w, 1743s, 1704(sh), 1678s, 1630m, 1502m, 1370s, 1220s(br.), 1086s. ¹H-NMR (200 MHz): 7.56-7.32 (m, 5 arom. H); 7.12 (dd, J=16.0, 4.5, H-C(4)); 6.77 (dd, J=16.0, 2.0, H-C(3)); 6.66 (d, J=8.5, NH); 5.49 (s, ArCH); 5.32-5.20 (m, H-C(5)); 5.18 (dd, J=4.5, 2.2, H-C(6)); 5.04 (ddd, J=10.5, 9.8, 5.2, H-C(8)); 4.40 (dd, J=10.5, 5.2, H-C(9)); 4.07 (dd, J=9.8, 2.2, H-C(7)); 3.65 (dd, J=10.5, 10.5, H-C(9)); 2.11 (s, CH₃); 2.07 (s, CH₃); 1.97 (s, CH₃); 1.56 (s, t-Bu).

⁵⁰) Addn. of 0.1% NEt₃ to the eluent or using SiO₂ impregnated with 2% NaHCO₃ led to faster moving, strongly UV-active by-products.

5-Acetamido-3,4,5-trideoxy-D-manno-2-nonulosonic Acid (=N-Acetyl-4-deoxyneuraminic Acid; **162**). A mixture of **163/164** (6.00 g, 10.4 mmol) and NaHCO_3 (600 mg, 7.1 mmol) in CHCl_3 (350 ml) was cooled to -60° and ozonized until the soln. turned blue⁵¹). It was purged with N_2 (10 min), treated with dimethylsulfide (1.15 ml), slowly warmed to -40° (2 h), treated with $(i\text{-Pr})_2\text{EtN}$ (3.5 ml), warmed to 0° (6 h) and stirred over night (TLC: $\text{AcOEt}/\text{hexane}$ 4:1). Extractive workup (0.1 M HCl , 10% NaHCO_3 , brine) gave **165** (5.9 g) as a slightly yellow foam. To a prehydrogenated suspension of 10% Pd/C (600 mg) in AcOEt (85 ml) was added a soln. of **165** in AcOEt (20 ml) and hydrogenated at r.t. for 24 h. TLC ($\text{AcOEt}/\text{hexane}$ 4:1, KMnO_4) indicated then complete hydrogenation of the olefinic double bond⁵²). The mixture was filtered through celite and the residue was carefully washed with a total of 100 ml AcOEt . Evaporation of the filtrates gave crude **166** as a colourless foam, which was dissolved in anh. MeOH (25 ml) and added (30 min) to a stirred, ice-cold soln. of K_2CO_3 (2.9 g) in $\text{MeOH}/\text{H}_2\text{O}$ 4:1 (200 ml). Stirring was continued at 0° (14 h) and then at r.t. (10 h). H_2O was added (40 ml), MeOH was removed and replaced by dioxane (40 ml). After addn. of Dowex 50 X 4 (H^+) (30 g) the mixture was stirred at r.t. over night. The resin was filtered off and washed twice with 30 ml H_2O . The combined filtrate and washings were extracted with CHCl_3 (100 ml). The aq. layer was freeze-dried to give crude **162** (brown residue, 3.5 g). A soln. of the residue in H_2O (5 ml) was brought to pH 9-10 by addn. of 1.0 M NaOH (ca. 10.5 ml) and the mixture was kept in the refrigerator (24 h). Compound **162** was purified by anion-exchange chromatography (160 ml Dowex 1X8 (HCOO^-); elution with 0.0 - 0.3 N aq. HCOOH). Fractions containing **162** were combined, concentrated, freeze-dried and finally dried over P_2O_5 (10^{-5} mbar, 2d) to give 4-deoxy-Neu5Ac **162** (1.60 g, 53%) as a microcrystalline solid, which decomposed at 162° . $[\alpha]_{\text{D}}^{25} = -47.6^\circ$ ($c = 1.1$, H_2O). $\text{pK}_{\text{a}} = 3.08$ (H_2O , 22°). IR(KBr): 3700-2300s, 2925m, 1723s, 1658s, 1529m. $^1\text{H-NMR}$ (400 MHz, D_2O): 4.13-3.93 (m, $\text{H-C}(5)$,

⁵¹) Ozonolysis in AcOEt at -70° gave approximately the same results.

⁵²) Shaking the suspension instead of stirring it shortens the reaction time. Cleavage of the benzylidene acetal was not observed.

H-C(6)); 3.83 (dd, J=11.8, 2.7, H-C(9)); 3.76 (dd, J=9.0, 6.3, 2.7, H-C(8)); 3.61 (dd, J=11.8, 6.3, H-C(9)); 3.57 (d, J=9.0, H-C(7)); 2.22-1.79 (m, 2 H-C(4), 2 H-C(3)); 2.00 (s, CH₃). ¹³C-NMR (50 MHz, D₂O): 174.57(s); 174.44(s); 94.89(s); 72.02(d); 70.71(d); 69.04(d); 63.82(t); 45.02(d); 31.17(t); 24.65(t); 22.54(q). FAB-MS: 294 ([M + 1]⁺). Anal. calc. for C₁₁H₁₉NO₈ (293.28): C 45.05, H 6.53, N 4.78; found: C 45.17, H 6.56, N 4.65.

tert-Butyl 5-Acetamido-7,9-O-benzylidene-3,4,5-trideoxy-D-manno-2-nonulosonate (168) and Methyl 5-Acetamido-7,9-O-benzylidene-3,4,5-trideoxy-D-manno-2-nonulosonate (169). A mixture of 163/164 (500 mg, 0.86 mmol) was ozonized, treated with (i-Pr)₂EtN and hydrogenated as described for 162. To an ice-cold soln. of the residue 166 in anh. MeOH (20 ml) was added dropwise a 0.5 M NaOMe/MeOH soln. (200 μl). The mixture was stirred at 0° (24 h). Additional NaOMe/MeOH (200 μl) was added and stirring was continued. After 48 h, the soln. was neutralized with 1.0 M AcOH in anh. MeOH. The solvent was evaporated. Flash chromatography of the residual foam (40 g SiO₂, CH₂Cl₂/EtOH 95:5) gave 168 (67 mg, 18%), 169 (81 mg, 24%) and 45 mg (12%) of a mixture of both. For analysis, 168 was crystallized from MeOH/Et₂O/hexane; 169 was crystallized from anh. MeOH/Et₂O.

Data of 168. M.p. 153-155°. [α]_D²⁵ = -53.0° (c = 1.0, MeOH). IR: 3435m(br.), 3350(sh); 2980m, 2935w, 2865w, 1730s, 1668s, 1510m, 1453m, 1390m, 1370s, 1147s, 1080s, 1028s, 928m. ¹H-NMR (400 MHz, (D₆)DMSO): 7.80 (d, J=9.0, NH); 7.51-7.27 (m, 5 arom. H); 6.16 (br.s, OH-C(2)); 5.32 (s, ArCH); 5.01 (br.s, OH-C(8)); 4.16 (dd, J=10.4, 5.3, H-C(9)); 4.06 (dd, J=10.5, 1.5, H-C(6)); 4.02-3.91 (m, H-C(5)); 3.79-3.70 (m, H-C(8); Addn. of D₂O: 3.73, ddd, J=10.0, 9.2, 5.3); 3.52 (dd, J=9.2, 1.5, H-C(7)); 3.47 (dd, J=10.5, 10.0, H-C(9)); 1.86-1.64 (m, 2 H-C(4), 2 H-C(3)); 1.81 (s, CH₃); 1.40 (s, t-Bu). ¹³C-NMR (50 MHz, (D₆)DMSO): 169.08(s); 168.19(s); 138.26(s); 128.04(d); 127.53(d); 125.97(d); 99.79(d); 93.59(s); 80.64(s); 79.50(d); 70.54(t); 68.62(d); 59.10(d); 42.65(d); 30.29(t); 27.43(q); 25.36(t); 22.65(q). CI-MS: 438 ([M + 1]⁺), 420, 384, 380, 366, 320. Anal. calc. for C₂₂H₃₁NO₈·2H₂O (473.53): C 55.80, H 7.45, N 2.97; found: C 55.64, H 7.62, N 2.78.

Data of 169. M.p. 215° (dec.). $[\alpha]_D^{25} = -73.4^\circ$ (c = 1.0, MeOH). IR(KBr): 3480m(br.), 3280m(br.), 2980w, 2905w, 2870w, 2840w, 1748s, 1646s, 1563m, 1388s, 1092s, 1080s, 1028m. $^1\text{H-NMR}$ (400 MHz, (D_6) DMSO): 7.82 (d, J=8.9, NH); 7.51-7.27 (m, 5 arom. H); 6.51 (br.s, OH-C(2)); 5.33 (s, ArCH); 5.13 (br.s, OH-C(8)); 4.16 (dd, J=10.5, 5.5, H-C(9)); 4.08 (dd, J=10.5, 1.2, H-C(6)); 4.06-3.94 (m, H-C(5)); 3.77-3.66 (m, H-C(8)); Addn. of D_2O : 3.70, ddd, J=10.0, 9.0, 5.5; 3.65 (s, CH_3); 3.53 (dd, J=9.0, 1.2, H-C(7)); 3.47 (dd, J=10.5, 10.0, H-C(9)); 1.82 (s, CH_3); 1.87-1.66 (m, 2 H-C(4), 2 H-C(3)). $^{13}\text{C-NMR}$ (25.2 MHz, (D_6) DMSO): 170.42(s); 168.13(s); 138.08(s); 128.01(d); 127.54(d); 125.89(d); 99.66(d); 93.71(s); 79.49(d); 70.54(t); 68.73(d); 59.01(d); 52.02(q); 42.64(d); 30.54(t); 25.43(t); 22.81(q). CI-MS: 396 ($[\text{M} + 1]^+$), 378, 336, 320, 290, 272. Anal. calc. for $\text{C}_{19}\text{H}_{25}\text{NO}_8$ (395.42): C 57.71, H 6.37, N 3.54; found: C 57.91, H 6.62, N 3.40.

tert-Butyl 5-Acetamido- 3,4,5-trideoxy-D- manno-2-nonulosonate (172). Similarly to 173, a soln. of 168 (115 mg, 0.26 mmol) in MeOH (5 ml) was hydrogenated in the presence of 10% Pd/C (25 mg). The crude product was crystallized from MeOH/Et₂O to give 172 (70 mg, 77%). M.p. 168-169° (dec.). $[\alpha]_D^{25} = -23.4^\circ$ (c = 0.9, H_2O). IR(KBr): 3480s(br.), 2985m, 2945m, 2885w, 1735s, 1662s, 1552m, 1374m, 1320m, 1300m, 1156m, 1085m, 1035m, 1024m. $^1\text{H-NMR}$ (400 MHz, D_2O): 4.04 (d, J=10.3, H-C(6)); 4.04-3.94 (m, H-C(5)); 3.86 (dd, J=11.7, 2.6, H-C(9)); 3.77 (ddd, J=8.8, 6.4, 2.6, H-C(8)); 3.63 (dd, J=11.7, 6.4, H-C(9)); 3.59 (d, J=8.8, H-C(7)); 2.01 (s, CH_3); 2.08-1.83 (m, 2 H-C(3), 2 H-C(4)); 1.51 (s, t-Bu); 2 singlets at 2.00 and 1.54 hint to the α -D-anomer. $^{13}\text{C-NMR}$ (50 MHz, D_2O): β -D-anomer: 174.39(s); 171.47(s); 94.74(s); 85.21(s); 71.83(d); 70.68(d); 68.88(d); 63.61(t); 44.91(d); 30.79(t); 27.42(q); 24.65(t); 22.42(q); α -D-anomer: 174.65(s); 85.42(d); 71.15(d); 69.80(d); 67.42(d); 27.66(q). CI-MS: 350 ($[\text{M} + 1]^+$), 294, 276. Anal. calc. for $\text{C}_{15}\text{H}_{27}\text{NO}_8$ (349.39): C 51.57, H 7.79, N 4.01; found: C 51.31, H 8.01, N 3.84.

Methyl 5-Acetamido- 3,4,5-trideoxy-D- manno-2-nonulosonate (173). A suspension of 169 (85 mg, 0.215 mmol) and 10% Pd/C (20 mg) in MeOH (5 ml) was hydrogenated at r.t. until TLC (CH₂Cl₂/MeOH 9:1) indicated the disappearance of 169. The catalyst was filtered off and washed with anh. MeOH. A soln. of the residue of the filtrates in H₂O (5 ml) was extracted with AcOEt. The aq. layer was freeze-dried to give 172 (65 mg, 98%), which was crystallized from MeOH/AcOEt/Et₂O; m.p. 163-164° (dec.). $[\alpha]_D^{25} = -38.0^\circ$ (c = 1.0, MeOH). IR(KBr): 3340s(br.), 2935w, 1740s, 1650s, 1535m, 1440w, 1375m, 1315m, 1288s, 1202w, 1152m, 1122m, 1075s, 1054m, 1022m. ¹H-NMR (400 MHz, D₂O): 4.05 (d, J=10.5, H-C(6)); 4.00 (ddd, J=10.5, 10.5, 4.2, H-C(5)); 3.85 (dd, J=11.8, 2.7, H-C(9)); 3.83 (s, CH₃); 3.76 (ddd, J=9.7, 6.4, 2.7, H-C(8)); 3.62 (dd, J=11.8, 6.4, H-C(9)); 3.57 (d, J=9.7, H-C(7)); 2.06-1.85 (m, 2 H-C(3), 2 H-C(4)); 2.01 (s, CH₃). ¹³C-NMR (50 MHz, (D₆)DMSO): 170.57(s); 170.40(s); 93.60(s); 71.34(d); 69.54(d); 68.99(d); 63.59(t); 52.05(q); 44.51(d); 30.67(t); 24.71(t); 22.46(q). CI-MS: 308 ([M + 1]⁺), 290, 248, 232. Anal. calc. for C₁₂H₂₁NO₈ (307.31): C 46.90, H 6.89, N 4.56; found: C 46.62, H 6.77, N 4.48.

tert-Butyl 5-Acetamido-3,4,5-trideoxy-D-glycero-D-talo- and -D-glycero-D-galacto-nononates (170). Similarly to 162, a soln. of 163/164 (250 mg, 0.433 mmol) was ozonized, treated with (i-Pr)₂EtN and hydrogenated to give crude 166 (200 mg). A soln. of the residue in MeOH (5 ml) was hydrogenated in the presence of 20% Pd(OH)₂ (30 mg) [201]. After 24 h, TLC (AcOEt/hexane 4:1) indicated the disappearance of the intermediate 166. The catalyst was filtered off and washed with MeOH. A soln. of the residue (170 mg) of the filtrates in anh. MeOH (4.5 ml) was treated with K₂CO₃ (4.5 mg) and stirred at r.t.; after 8 h, additional K₂CO₃ (3 mg) was added. Stirring was continued over night, TLC (CH₂Cl₂/MeOH 4:1) indicated then the disappearance of the intermediate. The soln. was neutralized (0.1 M AcOH/MeOH, 550 µl) and evaporated. FC of the residue (6 g SiO₂, CH₂Cl₂/MeOH 9:1) on a preconditioned column (CH₂Cl₂/MeOH 95:5) gave 170 (104 mg, 68%, 36 h P₂O₅ at 10⁻¹ mbar), which was crystallized from MeOH/Et₂O 1:1 and hexane. M.p. 97-105°. IR(KBr): 3370s(br.), 1733s, 1649s, 1556m, 1372m, 1170m, 1085m, 1035m. ¹H-NMR (200 MHz, CD₃OD): 4.07-3.38 (m, H-C(2), H-C(5)-H₂-C(9)); 2.14-1.50 (m, H₂-C(3), H₂-C(4)); 1.98 (s, CH₃); 1.48 (s, t-Bu).

For analysis, 170 was acetylated ($\text{Ac}_2\text{O}/\text{Py}$ 1:1, 0°) to give after FC ($\text{CHCl}_3/\text{MeOH}$ 200:1) 171 as a colourless foam. IR: 3430w, 3030w, 2980w, 2935w, 1740s, 1680s, 1500m, 1367s, 1216s(br.), 1152m, 1042m. ^1H -NMR (400 MHz): 5.41 (d, $J=5.3$, NH); 5.39 (dd, $J=8.0$, 3.0, H-C(7)); 5.13 (dd, $J=8.0$, 3.0, H-C(6)); 5.08 (ddd, $J=8.0$, 5.8, 3.0, H-C(8)); 4.84-4.79 (m, H-C(2)); 4.25 (dd, $J=12.5$, 3.0, H-C(9)); 4.27-4.19 (m, H-C(5)); 4.05 (dd, $J=12.5$, 5.8, H-C(9)); 2.13-2.05 (5 $\times$ CH_3); 1.97 (s, CH_3); 1.90-1.57 (m, $\text{H}_2\text{-C}(3)$, $\text{H}_2\text{-C}(4)$); 1.45 (s, t-Bu). ^{13}C -NMR (50 MHz): major product: 170.37(s); 170.19(s); 170.13(s); 169.83(s); 169.68(s); 168.86(s); 168.72(s); 82.12(s); 72.38(d); 71.59(d); 68.59(d); 68.23(d); 61.93(t); 48.01(d); 27.73(q); 27.30(t); 26.64 (t); 23.00(q); 20.61(q); 20.57(q); 20.51(q); 20.46(q); 20.41(q); minor product: 72.19(d); 71.73(d); 47.85(d); 27.24(t); 26.81(t). CI-MS: 562 ($[\text{M} + 1]^+$), 506. Anal. calc. for $\text{C}_{25}\text{H}_{39}\text{NO}_{13}$ (561.60): C 53.47, H 7.00, N 2.49; found: C 53.25, H 7.04, N 2.70.

6. Glycosidation of N-Acetyl-4-deoxyneuraminic Acid and Sialidase-assay

6.1. Synthesis of 4-Methylumbelliferyl 5-Acetamido-3,4,5-trideoxy- α -D-manno-2-nonulopyranosidonic Acid.

Methyl 5-Acetamido-2,7,8,9-tetra-O-acetyl-3,4,5-trideoxy- α - and - β -D-manno-2-nonulopyranosonate (174 and 175, resp.) and Methyl (Z)-5-acetamido-2,6,7,8,9-penta-O-acetyl-3,4,5-trideoxy-D-manno-2-nonenate (176).

A soln. of N-acetyl-4-deoxyneuraminic acid (162; 500 mg, 1.70 mmol) and CF_3COOH (50 μl) in anh. MeOH (15 ml) was stirred at r.t. (24 h), until TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 3:1) indicated the disappearance of 162. The soln. was evaporated to dryness to give crude methyl N-acetyl-4-deoxyneuraminate 173 (549 mg). To an ice-cold suspension of crude 173 in Ac_2O (7.5 ml) was added dropwise (30 min) pyridine (5.75 ml) [94]. The mixture was slowly warmed to r.t. (melting ice bath) and stirred for 3 days. TLC ($\text{CHCl}_3/\text{MeOH}$ 95:5) indicated then disappearance of 173. The solvent was removed, and the residue was co-evaporated with 3 x 20 ml toluene. Flash chromatography (75 g SiO_2 , CHCl_3 -MeOH 99:1) yielded the enol acetate 176 (52 mg, 6%) and the α -D-configured pentaacetate 174 (215 mg, 26.5%), while $\text{CHCl}_3/\text{MeOH}$ 80:1 yielded the β -D-configured pentaacetate 175 (505 mg, 62.5%). All 3 compounds were colourless foams.

Data of 174. $[\alpha]_D^{25} = +48.1^\circ$ ($c = 1.1$, CHCl_3). IR: 3435w, 2995w, 1746s, 1679m, 1502m, 1435w, 1369s, 1290m, 1240s(br.), 1220m, 1078s, 1044s. $^1\text{H-NMR}$ (200 MHz): 5.39 (dd, $J=6.5$, 2.6, H-C(7)); 5.30 (d, $J=9.5$, NH); 5.21 (ddd, $J=6.5$, 6.0, 2.8, H-C(8)); 4.71 (dd, $J=10.5$, 2.5, H-C(6)); 4.39 (dd, $J=12.5$, 2.8, H-C(9)); 4.08 (dd, $J=12.5$, 6.0, H-C(9)); 4.11-3.91 (m, H-C(5)); 3.73 (s, OCH_3); 2.28-1.88 (m, H_2 -C(3), H_2 -C(4)); 2.12, 2.10, 2.09, 2.04, 1.93 (5 x CH_3). $^{13}\text{C-NMR}$ (50 MHz): 170.70(s); 170.09(s); 170.02(s); 169.66(s); 169.07(s); 168.45(s); 95.80(s); 75.72(d); 70.29(d); 68.00(d); 62.30(t); 52.49(q); 43.64(d); 31.20(t); 26.17(t); 23.36(q); 20.84(q); 20.78(q); 20.72(q). CI-MS: 416 ($[\text{M} + 1]^+$ -AcOH). Anal. calc. for $\text{C}_{20}\text{H}_{29}\text{NO}_{12}$ (475.46): C 50.52, H 6.15, N 2.95; found: C 50.26, H 6.40, N 2.71.

Data of 175. $[\alpha]_D^{25} = -14.4^\circ$ ($c = 1.1$, CHCl_3). IR: 3435w, 2995w, 2955w, 1735s, 1681s, 1507m, 1438m, 1371s, 1290m, 1240s(br.), 1091m, 1040m, 1012m. $^1\text{H-NMR}$ (400 MHz): 5.39 (dd, $J=4.5, 1.8$, H-C(7)); 5.29 (d, $J=7.8$, NH); 5.06 (ddd, $J=7.0, 4.5, 2.5$, H-C(8)); 4.54 (dd, $J=12.5, 2.5$, H-C(9)); 4.14 (dd, $J=12.5, 7.0$, H-C(9)); 4.04-3.98 (m, H-C(5), H-C(6)); 3.77 (s, OCH_3); 2.25-1.74 (m, $\text{H}_2\text{-C}(3)$, $\text{H}_2\text{-C}(4)$); 2.13 (2x), 2.06, 2.03, 1.92 (5 x CH_3). $^{13}\text{C-NMR}$ (50 MHz): 170.54(s); 170.29(s); 169.56(s); 168.34(s); 167.40(s); 96.99(s); 73.74(d); 71.70(d); 68.37(d); 62.33(t); 52.87(q); 43.89(d); 30.31(t); 25.58(t); 23.34(q); 20.88(q); 20.71(q). CI-MS: 416 ($[\text{M} + 1]^+ \text{-AcOH}$). Anal. calc. for $\text{C}_{20}\text{H}_{29}\text{NO}_{12}$ (475.46): C 50.52, H 6.15, N 2.95; found: C 50.27, H 6.41, N 2.78.

Data of 176. $[\alpha]_D^{25} = +8.4^\circ$ ($c = 1.0$, CHCl_3). UV (EtOH): λ_{max} 213 nm (10500); (calc. [211]: 211 nm). IR: 3430w, 3030sh, 2995w, 2955w, 2925w, 2850w, 1745s, 1680s, 1500m, 1436m, 1368s, 1305m, 1216s(br.), 1130m, 1045m, 1026m. $^1\text{H-NMR}$ (200 MHz): 6.56 (dd, $J=8.5, 7.5$, H-C(3)); 5.54 (d, $J=10.0$, NH); 5.38 (dd, $J=8.4, 3.2$, H-C(7)); 5.23 (dd, $J=8.5, 3.2$, H-C(6)); 5.09 (ddd, $J=8.4, 5.8, 3.0$, H-C(8)); 4.50-4.32 (m, H-C(5)); 4.27 (dd, $J=12.5, 3.0$, H-C(9)); 4.05 (dd, $J=12.5, 5.8$, H-C(9)); 3.78 (s, OCH_3); 2.50-2.20 (m, $\text{H}_2\text{-C}(4)$); 2.27, 2.13, 2.11, 2.07, 2.06, 1.91 (6 x CH_3). $^{13}\text{C-NMR}$ (50 MHz): 170.56(s); 170.33(s); 170.16(s); 169.93(s); 169.54(s); 161.64(s); 140.44(s); 126.84(d); 70.96(d); 68.61(d); 68.19(d); 61.90(t); 52.56(q); 47.19(d); 29.67(q); 27.39(t); 23.09(q); 20.83(q); 20.75(q); 20.68(q); 20.36(q). EI-MS: 474 (1, $\text{M}^+ - 43$), 402(1), 360(2), 318(15), 259(10), 186(15), 139(25), 43(100). Anal. calc. for $\text{C}_{22}\text{H}_{31}\text{NO}_{13}$ (517.50): C 51.06, H 6.04, N 2.71; found: C 50.90, H 6.09, N 2.59.

Tetrabutylammonium Salt of 4-Methylumbelliferone (Tetrabutylammonium 4-Methyl-2-oxo-2-H-1-benzopyran-7-olate; 177). According to [215], to a soln. of NaOH (1.3 g, 32.5 mmol) in H_2O (30 ml) was added 4-methylumbelliferone (5.9 g, 30.4 mmol) and Bu_4NBr (10.7 g, 33.2 mmol). The mixture was shaken for 10 min. Extractive workup with CHCl_3 and crystallization from hot CHCl_3 gave 177 (2.0 g, 15%) as pale-green cubes. M.p. 177-179° (Lit. [215]: 170-172°, pale-green needles). In contrast to [215], where the tetrabutylammonium salt of 4-methylumbelliferone contained 2 coumarin residues per tetrabutylammonium group, 177 contained only 1

coumarin residue per tetrabutylammonium group as have been shown by $^1\text{H-NMR}$ spectrum. $^1\text{H-NMR}$ (80 MHz): 7.18 (d, $J=8.0$, H-C(5)); 6.42 (dd, $J=8.0$, 2.0, H-C(6)); 6.20 (d, $J=2.0$, H-C(8)); 5.51 (d, $J=0.5$, H-C(3)); 3.35-2.90 (m, 4 $\times$ $\text{CH}_2\text{-N}$); 2.26 (d, $J=0.5$, CH_3); 1.80-0.80 (m, 4 $\times$ $\text{CH}_2\text{CH}_2\text{CH}_3$).

Methyl (4-Methylumbelliferyl 5-Acetamido-7,8,9-tri-O-acetyl-3,4,5-tri-deoxy- α -D-manno-2-nonulopyranosid)onate (179) and Methyl 5-Acetamido-7,8,9-tri-O-acetyl-2,6-anhydro-3,4,5-trideoxy-D-manno-2-nonenonate (180). To a soln. of a 3:7 mixture of 174/175 (200 mg, 0.420 mmol) in dry Et_2O (12 ml) was added freshly distilled AcCl (0.8 ml). The mixture was cooled to -40°C ⁵³) and saturated with dry HCl .⁵⁴) The flask was stoppered and the clear soln. warmed to 0° within 5 h. TLC (AcOEt/acetone 92:8) indicated then the disappearance of 174/175 and showed two spots: a faster moving one corresponding to 180 and one for the glycosyl chloride. The solvent was evaporated, the residue dissolved in dry AcOEt (10 ml) and again evaporated to give a colourless foam, which was dried for 15 min. at 10^{-2} mbar. To a soln. of the residue in dry acetonitrile (8 ml) were added ground molecular sieves 3 Å (640 mg), 177 (460 mg, 1.050 mmol) and freshly prepared Ag_2CO_3 [283] (464 mg, 1.680 mmol). The mixture was stirred in the dark at r.t. for 18 h. The soln. was filtered through celite, washed with dry CHCl_3 (50 ml) and evaporated. The residue was dissolved in dry AcOEt ⁵⁵) (10 ml), stirred at r.t. (15 min), filtered through celite, washed with AcOEt and concentrated. FC (22 g SiO_2 , hexane/acetone 6:4) gave the dehydro sialic acid 180 (29 mg, 17%), the starting compound 174 (12 mg, 6%) and the 4-methylumbelliferyl glycoside 179 (187 mg, 75%). (TLC: hexane/acetone 1:1). All compounds were colourless foams.

⁵³) Usually 174/175 precipitated at this temperature.

⁵⁴) Freshly prepared from NaCl/conc.HCl and $\text{conc.H}_2\text{SO}_4$ according to [282].

⁵⁵) Excess of 177 is soluble in CHCl_3 , but not in AcOEt .

Data of 179. $[\alpha]_D^{25} = +64.8^\circ$ ($c = 1.0$, CHCl_3). IR: 3430w, 3030sh, 2995w, 2975w, 2950w, 1737s, 1683m, 1611s, 1558w, 1500m, 1440w, 1385m, 1367s, 1290m, 1220s(br.), 1142m, 1072s, 1040s, 855m. $^1\text{H-NMR}$ (400 MHz, $(\text{D}_6)\text{benzene}$): 7.29-7.23 (m, H-C(5'), H-C(6'), H-C(8')); 5.87 (d, $J=1.1$, H-C(3')); 5.85 (ddd, $J=7.7$, 6.0, 2.6, H-C(8)); 5.72 (dd, $J=7.7$, 2.0, H-C(7)); 5.25 (d, $J=9.5$, NH); 4.76 (dd, $J=10.7$, 2.0, H-C(6)); 4.65 (dd, $J=12.4$, 2.6, H-C(9)); 4.43 (dd, $J=12.4$, 6.0, H-C(9)); 4.31 (dddd, $J=11.0$, 10.7, 9.5, 4.0, H-C(5)); 3.30 (s, OCH_3); 2.37 (ddd, $J=13.7$, 4.0, 4.0, $\text{H}_{\text{eq}}\text{-C(3)}$); 2.18-1.60 (m, $\text{H}_{\text{ax}}\text{-C(3)}$, $\text{H}_{\text{eq}}\text{-C(4)}$); 2.14, 2.02, 1.90, 1.78 (4 x CH_3); 1.65 (d, $J=1.1$, $\text{H}_3\text{C-C(4')}$); 1.43-1.27 (m, $\text{H}_{\text{ax}}\text{-C(4)}$); attribution of the signals was achieved by double resonance spectroscopy. $^{13}\text{C-NMR}$ (50 MHz): 170.52(s); 170.15(s); 170.08(s); 169.73(s); 168.56(s); 160.98(s); 157.14(s); 154.41(s); 152.32(s); 125.61(d); 115.76(s); 115.39(d); 113.16(d); 107.21(d); 100.50(s); 75.64(d); 69.32(d); 67.72(d); 62.11(t); 52.82(q); 44.13(d); 32.68(t); 29.26(q); 26.76(t); 23.39(q); 20.97(q); 20.70(q); 18.65(q). EI-MS: 532 (2, $\text{M}^+ - \text{OAc}$), 416(25), 374(9), 356(18), 314(9), 254(7), 236(17), 195(8), 194(8), 177(25), 43(100). Anal. calc. for $\text{C}_{28}\text{H}_{33}\text{NO}_{13}$ (591.59): C 56.85, 5.62, N 2.37; found: C 56.59, H 5.88, N 2.17.

Data of 180. $[\alpha]_D^{25} = 23.6^\circ$ ($c = 1.0$, CHCl_3). UV (EtOH): λ_{max} 234 nm (6750) (calc. [211]: 240 nm). IR: 3435m, 3030w, 3000m, 2960m, 1740s, 1678s, 1505m, 1439m, 1371s, 1305m, 1230s(br.), 1130m, 1108s, 1045s. $^1\text{H-NMR}$ (200 MHz): 6.11 (dd, $J=4.0$, 4.0, H-C(3)); 5.71 (d, $J=9.0$, NH); 5.46 (dd, $J=5.7$, 3.7, H-C(7)); 5.25 (ddd, $J=7.0$, 3.7, 3.5, H-C(8)); 4.58 (dd, $J=12.0$, 3.5, H-C(9)); 4.45-4.35 (m, H-C(5)); 4.26 (dd, $J=12.0$, 7.0, H-C(9)); 4.18 (dd, $J=6.0$, 5.7, H-C(6)); 3.79 (s, OCH_3); 2.64 (ddd, $J=19.0$, 4.2, 4.0, H-C(4)); 2.21 (ddd, $J=19.0$, 4.2, 4.0, H-C(4)); 2.12 (s, CH_3); 2.07 (2 x CH_3); 1.97 (s, CH_3). $^{13}\text{C-NMR}$ (50 MHz): 170.49(s); 170.17(s); 169.83(s); 169.71(s); 162.11(s); 142.54(s, C(2)); 109.46(d, C(3)); 76.19(d); 70.67(d); 68.08(d); 61.69(t); 52.03(q); 41.63(d); 44.63(d); 26.64(t); 22.98(q); 20.68(q); 20.56(q); 20.48(q). CI-MS: 416 ($[\text{M} + 1]^+$), 356 ($[\text{M} + 1]^+ - \text{AcOH}$). Anal. calc. for $\text{C}_{18}\text{H}_{25}\text{NO}_{10}$: C 52.05, H 6.07, N 3.37; found: C 51.98, H 6.30, N 3.17.

Methyl (4-Methylumbelliferyl 5-Acetamido-3,4,5-trideoxy- α -D-manno-2-nonulopyranosid)onate (178). A) From 179. A soln. of 179 (65 mg, 0.110 mmol) in anh. MeOH (2.2 ml) was treated with 100 μ l of a 0.5 M NaOMe/MeOH soln.. After 4 h, TLC (hexane/acetone 4:6) indicated the disappearance of 9. The mixture was neutralized with 0.1 M AcOH in anh. MeOH and concentrated. FC (7 g SiO₂, CH₂Cl₂/MeOH 95:5) gave 178 (51 mg, >95%), as a colourless foam. $[\alpha]_D^{25} = +59.4^\circ$ (c = 1.0, CHCl₃). UV (MeOH): 202 (33'650), 282 (7'950), 313 (10'600). IR: 3660-3140m, 3435m, 3030w, 2995m, 2950m, 1728s, 1660m, 1612s, 1508m, 1388m, 1368m, 1264m, 1148m, 1130s, 1072s, 1037m, 1014m, 982m, 875w, 858m. ¹H-NMR (400 MHz): 7.52 (d, J=8.8, H-C(5')); 7.15 (dd, J=8.8, 2.4, H-C(6')); 7.02 (d, J=2.4, H-C(8')); 6.41 (d, J=8.3, NH); 6.20 (d, J=1.0, H-C(3')); 4.69 (d, J=4.2, OH); 4.14-3.58 (m, H-C(5)-H₂-C(9)); after addn. of D₂O: 4.73 (ddd, J=10.5, 10.5, 4.1, H-C(5)); 3.96 (dd, J=10.5, 1.4, H-C(6)); 3.93-3.84 (m, H-C(8), H-C(9)); 3.77 (dd, J=11.3, 4.1, H-C(9)); 3.63 (dd, J=9.0, 1.4, H-C(7)); 3.76 (s, OCH₃); 3.06 (s(br.), OH); 2.56 (ddd, J=13.0, 3.5, 3.5, H_{eq}-C(3)); 2.41 (d, J=1.0, H₃C-C(4')); 2.29-2.09 (m, H_{ax}-C(3), H_{eq}-C(4), OH); 2.03 (s, CH₃); 1.68-1.54 (m, H_{ax}-C(4)). ¹³C-NMR (50 MHz): 172.32(s); 169.06(s); 161.04(s); 156.88(s); 154.09(s); 152.62(s); 125.36(d); 117.40(d); 116.10(s); 113.17(d); 108.36(d); 101.30(s); 77.64(d); 70.34(d); 69.18(d); 64.13(t); 53.15(q); 44.32(d); 31.56(t); 26.30(t); 22.87(q); 18.51(q).

B) From 181. A soln. of 181 (20 mg, 0.044 mmol) in MeOH (1.5 ml) was treated with CH₂N₂ in Et₂O until a slightly yellow colour stayed. The solvent was removed and the residue flash chromatographed (5g SiO₂, cf. A)) to give 178 (16 mg, 77%). IR and ¹H-NMR spectra were indistinguishable from those obtained by de-O-acetylation of 179, with the exception of the chemical shifts of the OH signals.

4-Methylumbelliferyl 5-Acetamido-3,4,5-trideoxy- α -D-manno-2-nonulopyranosidonic Acid (181). To a soln. of 179 (100 mg, 0.169 mmol) in anh. MeOH (5 ml) were added 150 μ l of a 0.5 M NaOMe/MeOH soln. After 2 h at r.t., TLC (CH₂Cl₂/MeOH 4:1) indicated the disappearance of 179. The solvent was evaporated, the residue dissolved in H₂O (5 ml) and stirred at r.t. until the intermediate methyl ester 178 had disappeared as indicated by

TLC (AcOEt/MeOH/0.2 M aq. HCOOH 7:2:1). The soln. was acidified to pH 4 by addn. of Dowex 50 (H^+). The resin was filtered off, washed with H_2O (5 ml) and the filtrate was freeze-dried to give crude **181** (75 mg, 98%). The residue was dissolved in H_2O /MeOH (1:1) and purified by flash chromatography (8 g SiO_2 , AcOEt/MeOH/ H_2O 7:2:1). Fractions containing **181** were combined, concentrated, extracted with H_2O and freeze-dried to give **181** (60 mg, 78%; 72 h at 10^{-5} mbar over P_2O_5). $[\alpha]_D^{22} = +79.3^\circ$ ($c = 1.0$, H_2O). IR(KBr): 3420s, 1700m, 1612s, 1552m, 1435w, 1390m, 1370w, 1275w, 1135w, 1072m, 1040m, 1017w. 1H -NMR (400 MHz, D_2O): 7.71 (d, $J=8.5$, H-C(5')); 7.17 (dd, $J=8.5$, 2.2, H-C(6')); 7.16 (d, $J=2.2$, H-C(8')); 6.26 (d, $J=0.9$, H-C(3')); 4.16-4.02 (m, H-C(5), H-C(6)); 3.91 (ddd, $J=9.0$, 6.5, 2.5, H-C(8)); 3.89 (dd, $J=12.0$, 2.5, H-C(9)); 3.66 (dd, $J=12.0$, 6.5, H-C(9)); 3.63 (d, $J=9.0$, H-C(7)); 2.54 (ddd, $J=13.0$, 4.0, 4.0, H_{eq} -C(3)); 2.43 (d, $J=0.9$, H_3C -C(4')); 2.21-2.12 (m, H_{eq} -C(4)); 2.05 (ddd, $J=13.0$, 10.0, 4.5, H_{ax} -C(3)); 2.02 (s, CH_3); 1.69-1.56 (m, H_{ax} -C(4)). ^{13}C -NMR (50 MHz, D_2O): 174.68(s); 173.76(s); 164.61(s); 158.13(s); 156.39(s); 153.58(s); 126.41(d); 117.82(d); 115.92(s); 111.71(d); 107.97(d); 103.92(s); 76.56(d); 72.10(d); 68.96(d); 63.26(t); 44.77(d); 32.44(t); 26.70(t); 22.42(q); 18.33(q).

Sodium (4-Methylumbelliferyl 5-Acetamido-3,4,5-trideoxy- α -D-manno-2-nulopyranosid)onate (182). To a suspension of **178** (30 mg, 0.064 mmol) in H_2O (1 ml) were added 130 μ l of 0.5 M NaOMe/MeOH soln.. After 6 h at r.t., TLC (CH_2Cl_2 /MeOH 9:1) indicated the disappearance of **178**. The soln. was freeze-dried and the residue was chromatographed (6g SiO_2 , AcOEt/MeOH/2.5% aq. NEt_3 7:2:1). Fractions containing the triethylammonium salt were combined, concentrated and transformed into the sodium salt by passing it through Dowex 50 W x 4 (Na^+) (8 ml) to give after freeze-drying **182** (25 mg, 83%) as a microcrystalline solid, which decomposed at $142-144^\circ$. $[\alpha]_D^{25} = +74.9^\circ$ ($c = 1.0$, H_2O). 1H -NMR (400 MHz, D_2O): 7.68 (d, $J=8.6$, H-C(5')); 7.17 (dd, $J=8.6$, 1.7, H-C(6')); 7.15 (d, $J=1.7$, H-C(8')); 6.22 (s, H-C(3')); 4.12-4.03 (m, H-C(5), H-C(6)); 3.92 (ddd, $J=9.5$, 6.3, 2.0, H-C(8)); 3.90 (dd, $J=12.0$, 2.0, H-C(9)); 3.67 (dd, $J=12.0$, 6.3, H-C(9)); 3.63 (d, $J=9.5$, H-C(7)); 2.55 (ddd, $J=13.5$, 4.0, 3.5, H_{eq} -C(3)); 2.41 (s, H_3C -C(4')); 2.21-2.13 (m, H_{eq} -C(4)); 2.05 (ddd, $J=13.5$, 10.0, 4.5, H_{ax} -C(3)); 1.68-1.57 (m, H_{ax} -C(4)).

Methyl 2-acetoxy-2-propenoate (**183**) was prepared according to [214] in 36% yield. $^1\text{H-NMR}$ ([281], 200 MHz): 5.95 (d, $J=1.7$, H-C(3)) 5.39 (d, $J=1.7$, H-C(3)); 3.71 (s, $\text{CH}_3\text{-O}$); 2.14 (s, CH_3). $^{13}\text{C-NMR}$ (50 MHz): 168.69 (s); 161.71 (s, C(1)); 144.36 (s, C(2)); 113.81 (t, C(3)); 52.35 (q); 20.13 (q).

6.2. Sialidase (EC 3.2.1.18) Experiments⁵⁶⁾

The susceptibility of MU-4-deoxy-Neu5Ac **181** was assayed in 1 ml of 0.1 M NaOAc buffer containing 0.2 μmol of either **181** or MU-Neu5Ac **185** (synthesized according to Warner and O'Brien [284] with a slight modification by Berg et al. [285]) and 10 mU of sialidase. The sialidases tested were from Vibrio cholerae (Behringwerke, Marburg) and Arthrobacter ureafaciens (CalBiochem), respectively. In the case of the V. cholerae enzyme, the acetate buffer was adjusted to pH 5.5 and CaCl_2 was added to a final concentration of 1 mM, and in the case of the A. ureafaciens sialidase the pH was adjusted to pH 5.0. Blanks were run with both glycosides **181** and **185** containing no enzyme or containing inactivated (5 min heating at 100°) enzyme. The mixtures were incubated at 37° and 0.1 ml aliquots were withdrawn after various incubation times between 5 and 60 min followed by monitoring in a fluorimeter (365 nm for excitation and 450 nm for emission). The fluorescence of the blanks was subtracted from those of the assays containing sialidase.

To test a possible inhibitory effect of free N-acetyl-4-deoxyneuraminic acid (**162**) on sialidase activity, the following incubations were made: 1 ml incubation mixture of 0.1 M acetate buffer, pH 5, contained 5 mU of V. cholerae sialidase, 0.2 μmol MU-Neu5Ac **185**, and 1 μmol of the sodium salt

⁵⁶⁾ Investigated in the laboratories of Prof. Dr. R. Schauer, Biochemisches Institut der Universität Kiel, D-2300 Kiel.

of 4-deoxy-Neu5Ac. In blanks, the enzyme was omitted. After 0, 5, 10 and 15 min each 0.1 ml were withdrawn and measured fluorimetrically for liberated 4-methylumbelliferone.

The influence of MU-4-deoxy-Neu5Ac 181 on sialidase activity was checked by incubation in 1 ml 0.1 M acetate buffer, pH 5.5, 0.20 μ mol MU-Neu5Ac 185, 0.61 μ mol MU-4-deoxy-Neu5Ac 181 and 1 mU V. cholerae sialidase. For comparison, corresponding assays were made without MU-4-deoxy-Neu5Ac 181. In blanks sialidase was omitted. Liberated 4-methylumbelliferone was monitored after 0, 5, 10, 15, and 30 min by withdrawal of each 0.1 ml from this mixture.

7. Syntheses of 6-Amino-6-deoxy-sialic Acids

4,6-O-Benzylidene-1,2-dideoxy-3-O-formyl-1-nitro-D-ribo-hex-1-enopyranose (207) and 4,6-O-Benzylidene-2,3-dideoxy-D-erythro-hex-2-eno-6-lactone (208). To a soln. of 123 (1.0 g, 3.60 mmol) and Ph_3P (2.35 g, 9.00 mmol) in THF (20 ml) at 5° was added during 48 h a soln. of diethyl azodicarboxylate (1.41 ml, 9.0 mmol) and HCOOH (680 μl , 18.0 mmol) in THF (20 ml). When TLC (hexane/AcOEt 6:4) indicated the disappearance of 123, the mixture was diluted with AcOEt (40 ml), extracted with ice-cold 5% NaHCO_3 (50 ml), H_2O and brine. The solvent was removed and the residue flash chromatographed (200 g of SiO_2). Hexane/ Et_2O 7:3 eluted 207 (620 mg, 56%), hexane/ether 1:1 gave 208 (230 mg, 27%). An anal. sample of 207 was obtained by recrystallisation from AcOEt/hexane and one of 208 by recrystallisation from AcOEt/ Et_2O 1:2 and hexane.

Data of 207. M.p. $143\text{--}144^\circ$. $[\alpha]_{\text{D}}^{25} = +223^\circ$ ($c = 1.0$, CHCl_3). UV (CH_2Cl_2): $\lambda_{\text{max}} = 278$ (3620). IR: 3110w, 3020w, 2940w, 2870w, 1730s, 1665m, 1552s, 1468w, 1452w, 1382m, 1340s, 1271m, 1147s, 1100s, 1023m, 937m. $^1\text{H-NMR}$ (200 MHz): 8.13 (d, $J=1.1$, HCOO^-); 7.53-7.33 (m, 5 arom. H); 6.47 (d, $J=6.0$, $\text{H-C}(2)$); 5.84 (ddd, $J=6.0$, 4.0, 1.1, $\text{H-C}(3)$); 5.65 (s, ArCH); 4.69 (dd, $J=10.5$, 5.3, $\text{H-C}(6)$); 4.49 (ddd, $J=10.5$, 10.5, 5.3, $\text{H-C}(5)$); 4.09 (dd, $J=10.5$, 4.0, $\text{H-C}(4)$); 4.04 (dd, $J=10.5$, 10.5, $\text{H-C}(6)$). $^{13}\text{C-NMR}$ (50 MHz): 159.51(d); 154.81(s); 136.03(s); 129.45(d); 128.36(d); 126.01(d), 101.86(d); 96.94(d); 74.21(d); 67.96(d); 67.73(t); 61.01(d). Anal. calc. for $\text{C}_{14}\text{H}_{13}\text{NO}_7$ (307.27): C 54.73, H 4.26, N 4.56; found: C 54.49, H 4.39, N 4.82.

Data of 208. M.p. $133.0\text{--}133.5^\circ$ (Lit. [255]: $134\text{--}135^\circ$). $[\alpha]_{\text{D}}^{25} = +29.3^\circ$ ($c = 1.0$, CHCl_3) (Lit. [255]: $+26.5^\circ$ ($c = 1.0$, CHCl_3)). $^1\text{H-NMR}$ (400 MHz, (D_6)benzene): 7.47-7.45 (m, 2 arom. H); 7.17-7.11 (m, 3 arom. H); 6.15 (d(br.), $J=9.9$, $\text{H-C}(3)$); 5.47 (dd, $J=9.9$, 2.6, $\text{H-C}(2)$); 5.01 (s, ArCH); 3.89 (dd, $J=10.3$, 4.8, $\text{H-C}(6)$); 3.76 (ddd, $J=10.3$, 10.2, 4.8, $\text{H-C}(5)$); 3.55 (ddd, $J=10.2$, 2.6, 1.5, $\text{H-C}(4)$); 3.32 (dd, $J=10.3$, 10.3, $\text{H-C}(6)$). $^{13}\text{C-NMR}$ (50 MHz): 161.65(s); 146.85(d, C(3)); 136.23(s); 129.40(d); 128.30(d), 126.02(d); 120.55(d, C(2)); 102.18(d); 73.70(d); 72.58(d); 67.98(t).

4,6-O-Benzylidene-1,2-dideoxy-1-nitro-D-ribo-hex-1-enopyranose (211).

Similarly to 127 (Chap. 3), 207 (420 mg, 1.37 mmol) was deformylated with NaOMe/MeOH in THF/anh. MeOH to give after crystallization 211 (220 mg, 56%). FC (20 g of SiO₂, hexane/AcOEt 3:1) of the mother liquor gave further 211 (82 mg, 21%). M.p. 177-178°. $[\alpha]_D^{25} = +152.9^\circ$ (c = 1.1, CHCl₃). UV (CH₂Cl₂): $\lambda_{\max} = 279$ (3475). IR: 3575m, 3105w, 3020w, 2940w, 2870w, 1665m, 1550s, 1468w, 1453w, 1402w, 1383m, 1346s, 1334s, 1272m, 1143s, 1122s, 1102s, 1090s, 1026s, 996s. ¹H-NMR (200 MHz): 7.60-7.37 (m, 5 arom. H); 6.47 (d, J=5.8, H-C(2)); 5.72 (s, ArCH); 4.71 (dd, J=10.5, 5.3, H-C(6)); 4.66-4.56 (m, H-C(3); addn. of D₂O: 4.62 ,dd, J=5.8, 3.8); 4.52 (ddd, J=10.2, 10.2, 5.3, H-C(5)); 4.04 (dd, J=10.5, 10.2, H-C(6)); 3.93 (dd, J=10.2, 3.8, H-C(4)); 2.70 (d, J=1.8. OH). ¹³C-NMR (50 MHz): 154.06(s); 136.20(s); 129.64(d); 128.44(d); 126.14(d); 101.96(d); 100.23(d); 76.46(d); 67.71(t); 66.84(d); 60.33(d). Anal. calc. for C₁₃H₁₃NO₆ (279.26): C 55.91, H 4.69, N 5.02; found: C 56.14, H 4.57, N 4.95.

2-(N-4-Methoxybenzylidene)-amino-4,6-O-benzylidene-1,2-dideoxy-1-nitro-D-altropyranose (212). To an ice-cold soln. of 207 (2.76 g, 9.0 mmol) in THF (135 ml) were added 34 ml of a 25% soln. of NH₃ in H₂O. The mixture was stirred at 0° until 207 had disappeared (TLC: hexane/AcOEt 6:4, 4 h; giving first 211) and then at r.t. over night. THF was removed and the remaining aq. layer was freeze-dried. To a solution of the residue in anh. MeOH (9 ml) and dry benzene (90 ml) was added 4-Methoxy-benzaldehyde (2.2 ml, 1.8 mmol). The mixture was stirred at r.t. over night. The solvent was evaporated to 10 -15 ml and 212 (2.11 g, 57%) was crystallized by adding dry ether⁵⁷⁾. The mother liquor was concentrated and flash chromatographed (100 g of SiO₂). Hexane/THF 8:1 separated excess 4-Methoxy-benzaldehyd, while hexane/THF 7:3 eluted a mixture of 212/213 (1.20 g, 32%); 590 mg of 212 crystallized spontaneously at removal of the solvent. A sample for analysis was obtained by recrystallisation from AcOEt/hexane. M.p. 187-188° (dec.). $[\alpha]_D^{25} = -61.9^\circ$ (c = 1.0, THF). UV (CH₂Cl₂): $\lambda_{\max} = 283$ (18'810). IR (KBr): 3400m(br.), 1632m, 1602s, 1565s,

⁵⁷⁾ Sometimes the imin 212 precipitated directly from the reaction mixture.

1513m, 1377w, 1363m, 1306m, 1263s, 1172s, 1102s, 1045m, 983m, 834m, 761m. $^1\text{H-NMR}$ ((D_8) THF, 200 MHz): 8.51 (s, N=CH); 7.84-7.73 and 7.04-6.92 (4 arom. H, AA'BB'-system); 7.54-7.24 (5 arom. H); 5.68 (s, ArCH); 5.36 (s, H-C(1)); 4.92 (d, $J=3.0$, OH); 4.82 (ddd, $J=10.0$, 10.0, 5.0, H-C(5)); 4.53 (br.d, $J=4.0$, H-C(2)); 4.44 (dd, $J=10.0$, 5.0, H-C(6)); 4.30 (dd, $J=10.0$, 2.1, H-C(4)); 3.87 (dd, $J=10.0$, 10.0, H-C(6)); 3.90-3.80 (m, H-C(3)); 3.83 (s, CH_3O). $^{13}\text{C-NMR}$ ((D_8) -THF, 50 MHz): 165.14(d); 163.48(s); 139.02(s); 130.98(d); 129.60(s); 129.26(d); 128.43(d); 127.13(d); 114.65(d); 104.12(d); 102.67(d); 77.33(d); 73.90(d); 70.38(d); 69.43(t); 63.52(d); 55.57(q). Anal. calc. for $\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_7$ (414.43): C 60.86, H 5.35, N 6.76; found: C 61.06, H 5.30, N 6.50.

Equilibration of **212** into a mixture of **212/213**. A soln. of **212** (10 mg, 0.024 mmol) in anh. THF (1 ml) was treated with NEt_3 (3.3 μl , 0.024 mmol) and stored at 25° until $[\alpha]_{\text{D}}^{25}$ (-78.9° , $c = 1.0$) did not change any more (10 d). The solvent was removed and the residue was dried for 2 h at 10^{-2} mbar. The $^1\text{H-NMR}$ of the residue indicated a 85/15 mixture of **212** and **213**. $^1\text{H-NMR}$ of **213** (200 MHz, (D_8) THF): 8.23 (s, N=CH); 7.72-7.67 and 6.95-6.90 (4 arom. H, AA'BB'-system); 7.54-7.24 (5 arom. H); 5.97 (d, $J=2.0$, H-C(1)); 5.70 (s, ArCH); 3.80 (s, CH_3); H-C(2) - 2 H-C(6) were overlapped by the signals of **212**.

2-(N-4-Methoxybenzylidene)-amino-4,6-O-benzylidene-1,2-dideoxy-1-nitro-3-O-trifluoromethanesulfonate-D-altropyranose (**214**). To a soln. of **212** (100 mg, 0.24 mmol) in anh. CH_2Cl_2 (3ml) and pyridine (120 μl) at -30° was added trifluoromethanesulfonic acid anhydride (60 μl , 0.36 mmol). The mixture was slowly warmed to 0° and stirred (3 h) until TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 200:1) indicated the disappearance of **212**. Extractive workup (5% ice-cold NaHCO_3 , H_2O , brine) and purification of the residue by FC (15 g of SiO_2 , CH_2Cl_2) gave **214** (121 mg, 92%, colourless foam) as a 4:1 mixture of the α -/ β -D-anomers ($^1\text{H-NMR}$). IR: 1631m, 1602s, 1567s, 1558sh, 1508m, 1418m, 1303w, 1250m, 1165m, 1142s, 1113m, 936s. $^1\text{H-NMR}$ (200 MHz): 8.50 (s, 0.8 N=CH); 8.23 (s, 0.2 N=CH); 7.86-7.70 and 7.05-6.91 (4 arom. H, 2 AA'BB'-systems); 7.52-7.34 (m, 5 arom. H); 5.86 (d, $J=2.0$, 0.2 H-C(1)); 5.69 (s, ArCH); 5.34 (s, 0.8 H-C(1)); 5.28-5.22 (m, 0.2 H); 5.06-4.98 (m, 0.8 H-C(3)); 4.86 (d, $J=3.5$, 0.8 H-C(2)); 4.75 (ddd, $J=10.0$, 10.0,

5.0, 0.8 H-C(5)); 4.67-4.44 (m, 2.2 H; including a dd at 4.59, $J=10.0$, 5.0 for 0.8 H-C(6) and a dd at 4.57, $J=10.0$, 2.4 for 0.8 H-C(4)); 4.28 (ddd, $J=10.0$, 9.0, 4.5, 0.2 H-C(5)); 4.14 (dd, $J=10.0$, 10.0, 0.2 H-C(6)); 3.97 (dd, $J=10.0$, 10.0, 0.8 H-C(6)); 3.89 (s, 0.8 CH₃); 3.86 (s, 0.2 CH₃).

2-Acetamido-3-azido-4,6-O-benzylidene-1,2,3-trideoxy-1-nitro-D-manno-pyranose (215) and 2-Acetamido-4,6-O-benzylidene-1,2,3-trideoxy-1-nitro-D-threo-hex-3-enopyranose (216). Similarly to 214, 212 (1.00 g, 2.41 mmol) was treated with trifluoromethanesulfonic acid anhydride (600 μ l) to give after extractive workup crude 214 (1.40 g). To a soln. of crude 214 in dry benzene (20 ml) was added LiN₃ (235 mg, 4.8 mmol). HMPT (2.0 ml) was added dropwise to the mixture (30 min), which was stirred at r.t. over night. TLC (hexane/THF 6:4) indicated the disappearance of 214. The orange-brown soln. was diluted with AcOEt (100 ml) and extracted with 2 x 5% NaHCO₃ and brine. The residue (1.17 g) was dissolved in Et₂O/EtOH (99%) 1:3 (12.5 ml), cooled to 0° and treated with AcOH (1 ml) and tosylhydrazide (900 mg). The mixture was stirred at 0° (3 h) and then stored in the refrigerator over night. TLC (hexane/THF 1:1) indicated the conversion of the intermediate. Ac₂O (2.5 ml) was added to the ice-cold mixture, 5 h later N-acetylation was completed. Excess of Ac₂O was destroyed by adding ice (2.0 g) and stirring for 1 h (0°). The mixture was diluted with AcOEt (200 ml), extracted with 2 x 5% NaHCO₃ and brine and purified by FC (150 g of SiO₂, hexane/THF 7:3 to 1:1) to give 215 (707 mg, 81%) as a slightly yellow foam and 216 (100 mg, 13%) as an oil.

Data of 215. $[\alpha]_D^{25} = +58.2^\circ$ ($c = 1.1$, DMSO). IR: 3435w, 2995w, 2925w, 2865w, 2110s, 1688s, 1560s, 1495m, 1370s, 1255m, 1160m, 1120m, 1092s, 1028m, 1005m. ¹H-NMR (400 MHz): α -D-anomer: 7.50-7.35 (m, 5 arom. H); 5.84 (d, $J=5.7$, NH); 5.77 (d, $J=1.2$, H-C(1)); 5.66 (s, ArCH); 5.23 (ddd, $J=5.7$, 5.0, 1.2, H-C(2)); 4.46 (dd, $J=10.5$, 4.5, H-C(6)); 4.08 (dd, $J=10.0$, 5.0, H-C(3)); 3.97 (ddd, $J=10.0$, 9.0, 4.5, H-C(5)); 3.87 (dd, $J=10.0$, 9.0, H-C(4)); 3.84 (dd, $J=10.5$, 10.0, H-C(6)); 2.13 (s, CH₃). β -D-anomer: 6.00 (d, $J=9.8$, NH); 5.54 (d, $J=2.7$, H-C(1)); 5.31 (ddd, $J=9.8$, 5.2, 2.7, H-C(2)); 4.50 (dd, $J=10.5$, 4.5, H-C(6)); 4.10-3.76 (m, H-C(3), H-C(4), H-C(5), H-C(6) overlapped by the signals of the α -D-anomer); 2.09 (s, CH₃). ¹³C-NMR (50 MHz): 171.42(s); 136.06(s);

129.19(d); 128.23(d); 125.74(d); 103.55(d); 101.77(d); 76.20(d); 68.66(d); 67.68(t); 57.12(d); 49.71(d); 22.81(q). CI-MS: 317 ((M + 1)⁺ - HNO₂, 20), 107 (PhCH=OH⁺, 100). Anal. calc. for C₁₅H₁₇N₅O₆ (363.35): C 49.58, H 4.72, N 19.28; found: C 49.44, H 4.95, N 19.01.

Data of **216**. IR: 3445m, 2985w, 2875w, 1685s, 1560s, 1486s, 1455m, 1382s, 1370s, 1176s, 1082s, 1010m, 980m, 965m. ¹H-NMR (200 MHz): 7.51-7.29 (m, 5 arom. H); 5.65-5.51 (m, NH, ArCH, H-C(1)); 5.38-5.28 (m, H-C(2), H-C(3)); 4.83 (dd, J=10.5, 6.5, H-C(5)); 4.50 (dd, J=10.5, 6.5, H-C(6)); 3.72 (dd, J=10.5, 10.5, H-C(6)); 2.01 (s, CH₃). ¹³C-NMR (50 MHz): 169.60(s); 153.51(s, C(4)); 135.49(s); 129.84(d); 128.48(d); 126.14(d); 103.54(d); 102.75(d); 99.99(d); 69.38(t); 63.91(d); 45.46(d); 23.05(q).

5-Acetamido-6-azido-7,9-O-benzylidene-2,3,5,6-tetradecoxy-2-methylidene- α -D-manno-4-nonulo-4,8-pyranosono-1,4-lactone (217), tert-Butyl 5-Acetamido-6-azido-7,9-O-benzylidene-2,3,5,6-tetradecoxy-2-methylidene-D-manno-nonulopyranosonate (218) and tert-Butyl 5-Acetamido-6-azido-7,9-O-benzylidene-2,3,5,6-tetradecoxy-2-methylidene-D-manno-4-nonulosonate (219). To an ice-cold solution of **215** (1.10 g, 3.05 mmol) and tert-butyl 2(bromomethyl)prop-2-enoate (**54**, 1.01 g, 4.57 mmol) in THF (20 ml) was added dropwise DBU (960 μ l, 1 h). After 2 h TLC (AcOEt) indicated the disappearance of **215**. The mixture was diluted with AcOEt (20 ml) and extracted with H₂O, 5% NaHCO₃, brine. The residual oil was filtered through SiO₂ (150 g, hexane/AcOEt 1:4) to give the Michael addition product (1.365 g, 89%), which was dissolved in CH₃CN (55 ml) and citrate buffer (14 ml, pH 5.5), and stirred at r.t. (3 d, in the dark)⁵⁸. The mixture was diluted with AcOEt, extracted with H₂O, 5% NaHCO₃, brine and was then decolourized with charcoal. The slightly yellow solution was concentrated, crystallization from AcOEt/hexane gave **218** (834 mg, 58% from **215**). FC of the mother liquor (20 g of SiO₂, acetone/hexane 1:3) gave further **218/219** (78 mg, 5%), and the byproduct **217** (240 mg, 20%).

⁵⁸) The nitro group was not fully hydrolyzed after 4 days. Longer reaction times gave increased amounts of the by-product **217**.

Data of 218/219. An anal. sample was obtained by recrystallisation from AcOEt/hexane. M.p. 132-135° (dec.). $[\alpha]_D^{25} = -5.9^\circ$ (5 min.) $\rightarrow -27.8^\circ$ (48 h) (c = 1.0, DMSO). IR (KBr): 3410m, 3270m, 2975w, 2870w, 2105s, 1682s, 1650s, 1630m, 1540m, 1372s, 1160s, 1098s, 1070s, 1020s. ¹H-NMR (400 MHz, (D₆)acetone): 218; 7.50-7.34 (m, 5 arom. H); 7.31 (d, J=10.5, NH); 6.16 (d, J=1.6, 1 olef. H); 5.69 (br.s, 1 olef. H); 5.61 (s, ArCH); 5.60 (s, OH); 4.54 (dd, J=10.5, 4.1, H-C(5)); 4.16 (dd, J=10.5, 4.1, H-C(6)); 4.13-4.00 (m, H-C(7), H-C(8), H-C(9)); 3.74 (dd, J=10.5, 9.0, H-C(9)); 2.73 (d, J=14.3, H-C(3)); 2.68 (d, J=14.3, H-C(3)); 1.97 (s, CH₃); 1.48 (s, t-Bu). ¹H-NMR (200 MHz, (D₆)DMSO): 218/219 = 6:4 (24 h); 218; 8.00 (d, J=10.2, NH); 7.55-7.37 (m, 5 arom. H); 6.48 (s, OH); 6.09 (d, J=1.9, 1 olef. H); 5.71 (br.s, 1 olef. H); 5.66 (s, ArCH); 4.44-3.56 (m, 8 H); 1.94 (s, CH₃); 1.46 (s, t-Bu); 219; 8.51 (d, J=8.0, NH); 6.14 (d, J=1.6, 1 olef. H); 5.47 (br.s, 1 olef. H); 4.65 (dd, J=9.0, 8.0, H-C(5)); 1.97 (s, CH₃); 1.40 (s, t-Bu); all other signals were overlapped by the signals of 218. ¹³C-NMR (50 MHz, (D₆)DMSO): 218: 169.55(s); 164.98(s); 138.00(s); 135.85(s); 128.65(d); 127.95(d); 127.28(t); 126.19(d); 100.04(d); 99.17(s); 80.51(s); 79.60(d); 70.80(t); 60.85(d); 58.43(d); 55.66(d); 43.33(t); 27.54(q); 22.36(q); 219: 204.03(s); 169.84(s); 166.30(s); 137.55(s); 135.85(s); 129.03(d); 128.20(d); 128.07(t); 126.19(d); 101.30(d); 80.00(s); 76.39(d); 68.42(t); 64.31(d); 59.18(d); 52.24(d); 37.42(t); 27.72(q); 22.59(q). CI-MS: 447 ([M + 1]⁺ - N₂, 38), 107 (PhCH=OH⁺, 100). Anal. calc. for C₂₃H₃₀N₄O₇ (474.54): C 58.22, H 6.37, N 11.81; found: C 58.48, H 6.38, N 11.60.

Data of 217. An anal. sample was obtained by FC (CH₂Cl₂/MeOH 98:2) and subsequent crystallization from CH₂Cl₂/Et₂O. The compound decomposed at ca. 220° without melting until 300°. $[\alpha]_D^{25} = +44.0^\circ$ (c = 1.0, CHCl₃). IR: 3450m, 3005w, 2870w, 2120s, 1786s, 1690s, 1500m, 1372m, 1280m, 1260m, 1110s, 1098s, 1060m, 995s, 900m. ¹H-NMR (400 MHz): 7.52-7.33 (m, 5 arom. H); 6.34 (dd, J=3.4, 2.0, 1 olef. H); 5.72 (d, J=10.2, NH); 5.72 (dd, J=3.0, 2.0, 1 olef. H); 5.62 (s, ArCH); 4.76 (dd, J=10.2, 4.2, H-C(5)); 4.31 (dd, J=10.5, 4.5, H-C(6)); 4.28 (dd, J=10.5, 4.8, H-C(9)); 4.15 (ddd, J=10.0, 10.0, 4.8, H-C(8)); 3.75 (dd, J=10.5, 10.0, H-C(9)); 3.72 (dd, J=10.5, 10.0, H-C(7)); 3.00 (ddd, J=17.0, 3.4, 3.0, H-C(3)); 2.79 (ddd, J=17.0, 2.0, 2.0, H-C(3)); 2.15 (s, CH₃). attribution by sel. deco-

upl. experiments. ^{13}C -NMR (50 MHz, (D_6) acetone): 206.22(s); 138.12(s); 133.56(s); 129.57(d); 128.70(d); 126.84(d); 123.88(t), 105.58(s); 102.44(d); 77.18(d); 68.64(t); 67.20(d); 59.55(d); 53.32(d); 37.74(t); 22.68(q). CI-MS: 401 ($[\text{M} + 1]^+$, 100), 358 ($[\text{M} + 1]^+ - 43$, 61). Anal. calc. for $\text{C}_{19}\text{H}_{20}\text{N}_4\text{O}_6$ (400.41): C 56.99, H 5.03, N 14.00; found: C 57.21, H 5.21, N 13.79.

tert-Butyl 5-Acetamido-6-azido-7,9-O-benzylidene-2,3,5,6-tetradecoxy-2-methylidene-D-glycero-D-talo-nononate (220) and tert-Butyl 5-Acetamido-6-azido-7,9-O-benzylidene-D-glycero-D-galacto-nononate (221). To an ice-cold soln. of 218/219 (500 mg, 1.045 mmol) and HOAc^{59} (150 μl) in $\text{THF}/\text{H}_2\text{O}$ (4:1; 35 ml) was added NaBH_4 in small portions (ca 10 mg each batch) until TLC ($\text{AcOEt}/\text{hexane}$ 4:1) indicated the disappearance of 218/219 (2 h). The mixture was diluted with AcOEt (100 ml) and extracted with H_2O , aq. NaHCO_3 and brine. HPLC (Zorbax-Sil, $\text{AcOEt}/\text{hexane}$ 6:4) indicated a 84:16 mixture of 220 and 221. FC of the residue (30 g of SiO_2 impregnated with 2% NaHCO_3) gave 220 (380 mg, 76%; by elution with $\text{AcOEt}/\text{hexane}$ 4:1) and 221 (66 mg, 13%; by elution with AcOEt). Both compounds were colourless oils and as such less stable. THF soln. of 220 and 221, respectively, were stored in the refrigerator for several days.

Data of 220. $[\alpha]_{\text{D}}^{25} = -8.4^\circ$ ($c = 1.1$, CHCl_3). IR: 3420m(br.), 2975m, 2930w, 2860w, 2110s, 1678s, 1628w, 1507m, 1368s, 1148s, 1085s, 1072s, 1026m. ^1H -NMR (400 MHz): 7.53-7.36 (m, 5 arom. H); 6.51 (d, $J=9.4$, NH); 6.17 (d, $J=1.3$, 1 olef. H); 5.67 (br. s, 1 olef. H); 5.53 (s, ArCH); 4.51 (ddd, $J=9.4$, 9.0, 4.0, H-C(5)); 4.34 (dd, $J=10.5$, 5.2, H-C(9)); 4.26 (dd, $J=8.9$, 3.5, H-C(7)); 4.11-4.03 (m, H-C(6), H-C(8)); 3.95 (br. s, 1 OH); 3.74-3.68 (m, H-C(4); addn. of D_2O : ddd, $J=9.0$, 8.9, 2.2); 3.66 (dd, $J=10.5$, 10.0, H-C(9)); 3.34 (br. s, 1 OH); 2.66 (dd, $J=14.2$, 2.2, H-C(3)); 2.34 (dd, $J=14.2$, 8.6, H-C(3)); 1.86 (s, CH_3); 1.47 (s, t-Bu). ^{13}C -NMR (50 MHz): 170.95(s); 167.28(s); 138.03(s); 137.11(s); 129.33(d); 128.37(d); 127.77(t); 125.93(d); 101.12(d); 81.69(d); 81.32(s); 71.17(t); 61.59(d); 58.46(d); 55.47(d); 37.60(t); 27.90(q); 23.25(q). CI-MS: 448 ($[\text{M} + 1]^+ - \text{N}_2$).

⁵⁹⁾ Without HOAc a strong UV-active by-product was formed.

Data of 221. IR: 3425m(br.), 2990w, 2930w, 2855w, 2105s, 1678s, 1628sh, 1500m, 1369s, 1149sa, 1089s, 1071s, 1028m. ¹H-NMR (400 MHz): 7.56-7.30 (m, 5 arom. H); 6.38 (d, J=9.2, NH); 6.15 (d, J=1.3, 1 olef. H); 5.63 (d, J=0.6, 1 olef. H); 5.47 (s, ArCH); 4.43 (ddd, J=9.2, 7.2, 1.1, H-C(5)); 4.32 (dd, J=10.5, 5.2, H-C(9)); 4.16-4.10 (m, H-C(4)); 4.02 (ddd, J=10.0, 9.0, 5.2, H-C(8)); 3.96-3.86 (m, OH); 3.82 (dd, J=9.0, 3.0, H-C(7)); 3.78 (dd, J=7.2, 3.0, H-C(6)); 3.61 (dd, J=10.5, 10.0, H-C(9)); 3.48-3.29 (m, OH); 2.49-2.41 (m, 2 H-C(3)); 2.03 (s, CH₃); 1.49 (s, t-Bu).

tert-Butyl 5-Acetamido-2,6-anhydro-7,9-O-benzylidene-2-deoxy-3,5-di-deoxy-2-imino-D-glycero-D-talo-nonulosonate (222) and tert-Butyl 5-Acetamido-2-amino-2,6-anhydro-7,9-O-benzylidene-2,3,5-trideoxy-D-glycero-D-talo-non-2-enonate (223). To a soln. of 220 (380 mg, 0.797 mmol) in CH₂Cl₂ (50 ml) was added NaHCO₃ (38 mg). The mixture was cooled to -78 ° and ozonized until the colour turned blue. The soln. was purged with O₂ (2 min) and N₂ (10 min), and mixed with Ph₃P (167 mg, 0.8 eq.) dissolved in CH₂Cl₂ (1 ml). After warming to r.t. (30 min) the solvent was removed and the residue was dissolved in MeOH (12 ml). To the stirred soln. was added HCOO⁻NH₄⁺ (243 mg) and Pd-C (10%, 110 mg); 10 min later additional Pd-C (150 mg) was added and stirring was continued for 30 min (r.t.). TLC (CH₂Cl₂/MeOH 9:1) showed then a main spot. The mixture was filtered through celite, washed with AcOEt (50 ml), and the filtrate was extracted with sat. Na₂CO₃ and brine. FC (25 g SiO₂, CH₂Cl₂/MeOH 95:5) gave a mixture of the imine 222 and the enamine 223 (324 mg, 94%) as a slightly yellow foam. IR: 3420m (br.), 2985m, 2930w, 2860w, 1710s, 1665s, 1510m, 1394m, 1370s, 1285s, 1156s, 1072s, 1028s. ¹H-NMR (400 MHz, D₃COD): 7.49-7.27 (m, 5 arom. H); 5.50 (d, J=5.0, 0.45 H-C(3) of 223); 5.48 (s, 0.45 ArCH of 223); 5.45 (s, 0.55 ArCH of 222); 4.40-4.20 (m, 3.45 H); 4.09-4.05 (m, 0.55 H); 3.84-3.57 (m, 3.0 H); 2.78 (ddd, J=19.0, 2.0, 2.0, 0.55 H-C(3) of 222); 2.57 (dddd, J=19.0, 3.5, 3.0, 1.0, H-C(3) of 222); 2.03 (s, 0.55 CH₃ of 222); 2.02 (s, 0.45 CH₃ of 223); 1.51 (s, 0.55 t-Bu of 222); 1.49 (s, 0.45 t-Bu of 223). ¹³C-NMR (50 MHz, D₃COD): 173.25(s); 173.10(s); 164.65(s); 164.57(s); 161.80(s, C(2) of 222); 139.28(s);

1398.13(s); 136.64(s, C(2) of 223); 129.57(d); 129.22(d); 128.87(d); 128.84(d); 127.26(d); 127.19(d); 103.96(d, C(3) of 223); 102.23(d); 101.94(d) 83.41(s); 82.74(s); 82.24(d); 81.11(d); 72.31(t); 65.33(d); 64.07(d); 62.18(d); 61.32(d); 58.36(d); 50.99(d); 49.64(d); 47.57(d); 36.34 (t, C(3) of 222); 28.19(q); 28.10(q); 22.83(q); 22.79(q). CI-MS: 419, 417, 415, 358. Anal. calc. for $C_{22}H_{30}N_2O_7$ (434.50): C 60.82, H 6.96, N 6.45; found: C 60.59, H 7.20, N 6.28.

tert-Butyl 5-Acetamido-2-amino-2,6-anhydro-7,9-O-benzylidene-2,3,5-trideoxy-D-erythro-L-allo-nononate (224) and tert-Butyl 5-Acetamido-2-amino-2,6-anhydro-7,9-O-benzylidene-2,3,5-trideoxy-D-erythro-L-altro-nononate (225)
To a soln. of 222/223 (305 mg, 0.703 mmol) in AcOEt (15 ml) and benzene (3 ml) was added PdC (10%, 270 mg). The mixture was hydrogenated at r.t. for 17 h. Additional PdC (10%, 70 mg) was added and hydrogenation was continued (5 h). TLC ($CH_2Cl_2/MeOH$ 95:5) indicated then the disappearance of 222/223. The mixture was filtered through celite, washed with MeOH and concentrated. FC (30 g of SiO_2 , $CH_2Cl_2/MeOH$ 95:5) gave 225 (59 mg, 19%), which crystallized from ether, and 224 (229 mg, 75%), which solidified at co-evaporation with benzene.

Data of 224. $[\alpha]_D^{25} = -75.5^\circ$ (c = 1.1, MeOH). IR: 3435m, 3320m, 2985m, 2935m, 2875m, 1729s, 1670s, 1505m, 1455w, 1371s, 1310m, 1292m, 1148s, 1090s, 1030s, 980m. 1H -NMR (400 MHz, CD_3OD): 7.54-7.28 (m, 5 arom. H); 5.42 (s, ArCH); 4.23 (dd, J=10.5, 5.3, H-C(9)); 4.08-4.05 (m, H-C(4)); 4.00 (ddd, J=10.0, 9.5, 5.3, H-C(8)); 3.96 (dd, J=10.8, 2.8, H-C(5)); 3.69 (dd, J=12.0, 3.0, H-C(2)); 3.56 (dd, J=9.5, 1.2, H-C(7)); 3.54 (dd, J=10.5, 10.0, H-C(9)); 3.31 (dd, J=10.8, 1.2, H-C(6)); 2.05 (ddd, J=13.0, 3.5, 3.0, H_{eq} -C(3)); 2.01 (s, CH_3); 1.54 (ddd, J=13.0, 12.0, 1.5, H_{ax} -C(3)); 1.45 (s, t-Bu). ^{13}C -NMR (50 MHz, CD_3OD): 174.70(s); 173.10(s); 139.49(s); 129.71(d); 128.98(d); 127.40(d); 102.32(d); 82.45(s); 80.97(d); 72.47(t); 67.75(d); 61.07(d); 53.84(d); 51.63(d); 50.54(d); 38.85(t); 28.25(q); 22.81(q). CI-MS: 437 ($[M + 1]^+$, 100). Anal. calc. for $C_{22}H_{32}N_2O_7$ (436.52): C 60.53, H 7.39, N 6.42; found: C 60.65, H 7.53, N 6.23.

Data of 225. M.p. 210-211° (dec.). $[\alpha]_D^{25} = -92.0^\circ$ (c = 1.1, MeOH). IR: 3420m, 3350sh, 2985m, 2935w, 2870w, 1725s, 1666s, 1510m, 1392m, 1371s, 1151s, 1080s, 1030m. $^1\text{H-NMR}$ (400 MHz, CD_3OD): 7.53-7.28 (m, 5 arom. H); 5.43 (s, ArCH); 4.25 (dd, J=10.5, 5.3, H-C(9)); 4.10 (dd, J=9.6, 2.8, H-C(5)); 4.01-3.98 (m, H-C(4)); 3.96 (ddd, J=10.0, 9.5, 5.3, H-C(8)); 3.63 (dd, J=9.6, 1.9, H-C(6)); 3.59 (dd, J=9.5, 1.9, H-C(7)); 3.55 (dd, 10.5, 10.0, H-C(9)); 3.50 (dd, J=6.0, 3.0, H-C(2)); 2.28 (ddd, J=14.0, 4.6, 3.0, $\text{H}_{\text{eq}}\text{-C(3)}$); 1.99 (s, CH_3); 1.89 (ddd, J=14.0, 6.0, 2.4, $\text{H}_{\text{ax}}\text{-C(3)}$); 1.48 (s, t-Bu). $^{13}\text{C-NMR}$ (50 MHz, CD_3OD): 174.86(s); 172.91(s); 139.47(s); 129.56(d); 128.88(d); 127.38(d); 102.24(d); 82.02(s); 81.43(d); 72.54(t); 67.83(d); 61.53(d); 53.32(d); 50.68(d); 50.57(d); 35.60(t); 28.27(q); 22.84(q). CI-MS: 437 ($[\text{M} + 1]^+$, 100). Anal. calc. for $\text{C}_{22}\text{H}_{32}\text{N}_2\text{O}_7$ (436.52): C 60.53, H 7.39, N 6.42; found: C 60.42, H 7.59, N 6.37.

tert-Butyl 5-Acetamido-2-amino-2,6-anhydro-7,9-O-benzylidene-2,3,5-trideoxy-D-erythro-L-gluco-nononate (226). Similarly to 220, a soln. of 221 (130 mg, 0.272 mmol) in CH_2Cl_2 (18 ml) was ozonized, treated with PPh_3 (58 mg) and concentrated. The residue was dissolved in MeOH (15 ml), Pd/C (10%, 1.1 g)⁶⁰ was added and the mixture was hydrogenated⁶¹. After 5-10 min, TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1) showed a main spot, indicating the disappearance of the ozonolysis products. The mixture was filtered through celite, washed with MeOH (20 ml) and evaporated. FC (20 g of SiO_2 , $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95:5) gave 226 (68 mg, 58%), which solidified at solvent evaporation. $[\alpha]_D^{25} = -43.5^\circ$ (c = 1.0, MeOH). IR: 3430m, 3320m, 2980m, 2930m, 2870m, 1730s, 1655s, 1515w, 1455m, 1370s, 1150s, 1070s, 1030m. $^1\text{H-NMR}$ (400 MHz, D_3COD): 7.55-7.31 (m, 5 arom. H); 5.43 (s, ArCH); 4.23 (dd, J=10.5, 5.0, H-C(9)); 3.97 (ddd, J=10.5, 9.5, 5.0, H-C(8));

⁶⁰) We suspect that a part of the catalyst was poisoned by the formed amine.

⁶¹) Reduction of the azido function with $\text{HCOO}^-\text{NH}_4^+/\text{PdC}$ as described for 220 gave multicomponent mixtures, which combined to 226 at succeeding hydrogenation (H_2 , Pd/C).

3.75 (dd, $J=10.3, 10.0$, H-C(5)); 3.64-3.56 (m, H-C(4)); 3.58 (dd, $J=9.5, 1.0$, H-C(7)); 3.53 (dd, $J=10.5, 10.5$, H-C(9)); 3.30 (dd, $J=11.8, 2.8$, H-C(2)); 2.89 (dd, $J=10.3, 1.0$, H-C(6)); 2.24 (ddd, $J=12.5, 4.5, 2.8$, H_{eq}-C(3)); 2.02 (s, CH₃); 1.46 (s, t-Bu); 1.27 (ddd, $J=12.5, 11.8, 10.0$, H_{ax}-C(3)); the signals were attributed by sel. decoupl. experiments. ¹³C-NMR (50 MHz, CDCl₃): 172.73(s); 171.55(s); 137.44(s); 128.85(d); 128.08(d); 126.10(d); 101.07(d); 81.68(s); 79.45(d); 72.40(d); 71.15(t); 60.69(d); 56.58(d); 55.39(d); 54.08(d); 38.42(t); 27.87(q); 23.17(q). CI-MS: 437 ((M + 1)⁺, 100). Anal. calc. for C₂₂H₃₂N₂O₇ (436.52): C 60.53, H 7.39, N 6.42; found: C 60.58, H 7.59, N 6.27.

5-Acetamido-2-amino-2,6-anhydro-2,3,5-trideoxy-D-erythro-L-allo-nononic acid (227). A soln. of 224 (100 mg, 0.230 mmol) in trifluoro acetic acid (3 ml) was stirred at r.t. until TLC (CHCl₃/MeOH 9:1 and 3:1) indicated the disappearance of 224 (3 h). The solvent was removed, the residue dissolved in H₂O (10 ml) and extracted with CH₂Cl₂. The aqueous layer was freeze-dried to give crude 227 (95 mg), which was dissolved in H₂O (1 ml), basified with 0.5 N NaOH (pH 9) and chromatographed on Dowex 1X8 (HCOO⁻, 10 g, elution with 0 - 0.3 N HCOOH, linear). Fractions containing 227 were combined and freeze-dried to give pure 227 (30 mg, 44%, 2d at 10⁻⁵ mbar). The later eluted fractions (70 pieces a 5 ml) were combined, freeze-dried and again chromatographed on Dowex 1X8 (HCOO⁻) giving further 227 (20mg, 28%). $[\alpha]_D^{25} = -74.9^\circ$ (c = 1.0, H₂O). IR (KBr): 3700-2300s, 1630s, 1550m. ¹H-NMR (400 MHz, D₂O): 4.26 (dd, $J=11.0, 2.5$, H-C(5)); 4.25-4.20 (m, H-C(4)); 4.03 (dd, $J=13.0, 3.0$, H-C(2)); 3.94 (ddd, $J=6.0, 5.2, 4.5$, H-C(8)); 3.83 (d, $J=6.0$, H-C(7)); 3.79 (dd, $J=11.5, 4.5$, H-C(9)); 3.75 (d, $J=11.0$, H-C(6)); 3.67 (dd, $J=11.5, 5.2$, H-C(9)); 2.39 (ddd, $J=15.0, 3.5, 3.0$, H_{eq}-C(3)); 2.08 (ddd, $J=15.0, 13.0, 1.5$, H_{ax}-C(3)); 2.07 (s, CH₃); attribution by sel. decoupl. experiments. ¹³C-NMR (50 MHz, D₂O): 174.39(s); 173.65(s); 72.46(d); 65.91(d); 64.91(d); 62.15(t); 54.00(d); 53.81(d); 48.00(d); 32.19(t); 22.00(q). FAB-MS: 293 ([M + 1]⁺). Anal. calc. for C₁₁H₂₀N₂O₇ × 1 H₂O (310.32): C 42.58, H 7.15, N 9.03; found: C 42.30, H 7.18, N 9.01.

5-Acetamido-2-amino-2,6-anhydro-2,3,4,5-tetradexo-D-glycero-D-talo-nononic acid (228). Similarly to 224, 225 (40 mg, 0.092 mmol) was deprotected with trifluoro acetic acid to give after ion-exchange chromatography (6 g Dowex 1X8 (HCOO⁻) the deoxygenated compound 228 (11 mg, 43%). $[\alpha]_D^{25} = -38.8^\circ$ (c = 1.0, H₂O). ¹H-NMR (400 MHz, D₂O): 4.14 (ddd, J=11.0, 11.0, 4.2, H-C(5)); 3.93 (ddd, J=5.5, 5.0, 4.0, H-C(8)); 3.87 (dd, J=5.0, 1.0, H-C(7)); 3.77 (dd, J=11.5, 4.0, H-C(9)); 3.75 (dd, J=13.0, 3.2, H-C(2)); 3.65 (dd, J=11.5, 5.5, H-C(9)); 3.44 (dd, J=11.0, 1.0, H-C(6)); 2.34 (dddd, J=14.0, 7.0, 3.5, 3.2, H_{eq}-C(3)); 2.18 (dddd, J=13.0, 7.0, 4.2, 3.0, H_{eq}-C(4)); 2.03 (s, CH₃); 1.85 (dddd, J=14.0, 13.0, 12.0, 3.0, H_{ax}-C(3)); 1.72 (dddd, J=13.0, 12.0, 11.0, 3.5, H_{ax}-C(4)); attribution by sel. decoupl. experiments. ¹³C-NMR (100.6 MHz, D₂O): 177.20; 176.22; 75.35; 68.73; 64.93; 62.50; 62.03; 47.51; 31.87; 27.71; 24.85. FAB-MS (NOBA): 299 ([M + Na]⁺), 277 ([M + 1]⁺).

5-Acetamido-2-amino-2,6-anhydro-2,3,5-trideoxy-D-erythro-L-altro-nononic acid (229). Similarly to 226, 225 (50 mg, 0.115 mmol) was deprotected by treating it subsequently with aq. NaOH and aq. HCl to give after (the second) ion-exchange chromatography 229 (33 mg, 92%, 2d at 10⁻⁵ mbar over P₂O₅) as the monohydrate. $[\alpha]_D^{25} = -41.0^\circ$ (c = 1.0, H₂O). IR (KBr): 3700-2300s, 1630s, 1560m. ¹H-NMR (400 MHz, D₂O): 4.18 (dd, J=11.5, 2.0, H-C(5)); 4.12 (d, J=11.5, H-C(6)); 4.10-4.07 (m, H-C(4)); 3.99-3.93 (m, H-C(8), H-C(2)); 3.84 (d, J=5.0, H-C(7)); 3.76 (dd, J=11.5, 5.0, H-C(9)); 3.72 (dd, J=11.5, 6.5, H-C(9)); 2.59 (ddd, J=15.0, 4.0, 1.5, H_{eq}-C(3)); 2.17 (ddd, J=15.0, 7.0, 2.2, H_{ax}-C(3)); 2.05 (s, CH₃). ¹³C-NMR (50 MHz, D₂O): 174.45(s); 174.05(s); 73.60(d); 65.67(d); 64.82(d); 62.59(t); 52.76(d); 51.21(d); 48.33(d); 30.48(t); 22.24(q). FAB-MS: 293 ([M + 1]⁺). Anal. calc. for C₁₁H₂₀N₂O₇ × 1 H₂O (310.32): C 42.58, H 7.15, N 9.03; found: C 42.43, H 7.21, N 8.98.

5-Acetamido-2-amino-2,6-anhydro-2,3,5-trideoxy-D-erythro-L-gluco-nononic acid (230). A soln. of 226 (75 mg, 0.171 mmol) in MeOH (0.25 ml) and 0.5 N NaOH (1.5 ml) was stirred over night at r.t. TLC (CHCl₃/MeOH 4:1 and i-PropOH/MeOH/0.3 N HCOOH 6:1:3) indicated then the disappearance of 226. The mixture was chromatographed on Dowex 1X8 (HCOO⁻; 9 g, elution with 0 - 0.3 N HCOOH) to give after freeze-drying the 7,9-O-benzylidene

protected amino acid (64 mg, 98%), which was dissolved in 1 N HCl (3 ml) and stirred at r.t. After 9 h the benzylidene acetal had been hydrolyzed as indicated by TLC. The mixture was diluted with H₂O (3 ml) and extracted with CH₂Cl₂. The aqueous layer was freeze-dried, the crude 230 was dissolved in 0.5 N NaOH and purified by ion-exchange chromatography (12 g Dowex 1x8 (HCOO⁻), elution with 0 - 0.5 N HCl⁶²). Fractions containing the product were combined and freeze-dried to give 230 (52 mg, 98%, 2d at 10⁻⁵ mbar) as the monohydrate. $[\alpha]_D^{25} = -20.3^\circ$ (c = 0.7, H₂O). IR (KBr): 3700-2400s, 1630s, 1555m. ¹H-NMR (400 MHz, D₂O): 4.03 (dd, J=11.0, 10.0, H-C(5)); 3.95-3.89 (m, H-C(4), H-C(8)); 3.85 (dd, J=13.0, 3.0, H-C(2)); 3.85 (d, J=6.0, H-C(7)); 3.78 (dd, J=11.5, 4.5, H-C(9)); 3.65 (dd, J=11.5, 5.2, H-C(9)); 3.49 (d, J=11.0, H-C(6)); 2.60 (ddd, J=13.0, 4.5, 3.0, H_{eq}-C(3)); 2.09 (s, CH₃); 1.86 (ddd, J=13.0, 13.0, 11.0, H_{ax}-C(3)); attribution by sel. decoupl. experiments. ¹³C-NMR (50 MHz, D₂O): 175.48(s); 172.48(s); 72.89(d); 69.23(d); 66.16(d); 62.36(t); 57.98(d); 57.56(d); 51.65(d); 33.20(t); 22.49(q). FAB-MS: 293 ([M + 1]⁺). Anal. calc. for C₁₁H₂₀N₂O₇ × 1 H₂O (310.32): C 42.58, H 7.15, N 9.03; found: C 42.32, H 7.40, N 9.25.

62) Elution with aq HCOOH instead of aq. HCl led to diminished yield of 230, due to partially very slow release of 230 from the resin (cf. also 227).

8. SUMMARY

This work describes the elaboration of the basis of a synthesis of N-acetylneuraminic acid using 1-deoxy-1-nitro-aldoses and the synthesis of

- Neu5Ac and 4-epi-Neu5Ac
- 4-deoxy-Neu5Ac
- the 4-methylumbelliferyl α -D-glycoside of 4-deoxy-Neu5Ac
- 6-amino-6-deoxy-sialic acids.

Radical denitration with Bu_3SnH of the pyranose derivatives 64, 65, 70, and 71 gave in good yields exclusively the 'C-glycosides' 66 and 72, respectively (Scheme 11). This behaviour was compared to the one of the cyclohexyl derivatives 76, 77, 80, and 81 giving a 4:1 mixtures of 78/79 and 82/83, respectively. In the furanose series a divergent behaviour was observed for the D-mannose-derived nitro ethers 86 and 88 and the D-ribose-derived nitro ethers 91 and 92, respectively, in that the former two gave isomerically homogeneous reduction products (87 and 89, respectively; Scheme 13) and the latter a 1:1 mixture of the diastereoisomers 93 and 94 (Scheme 14). This was explained on the basis of the stereoelectronic and steric effects of the ring O-atom.

By analogy to a known procedure (used for the preparation of protected 1-deoxy-1-nitro-aldoses), the unprotected 1-deoxy-1-nitro-mannopyranose 115 was prepared. Treatment of 115 with 2-methoxypropene gave the isopropylidene derivatives 116/117, which were converted into the 1-C-nitroglycal 118 (Scheme 16). Similarly to 118, the differentially protected nitro olefins 121, 123, 127 and 129 have been prepared (Scheme 18). The β -addition of NH_3 to 1-C-nitroglycals was followed by an O $\rightarrow$ N acetyl migration to give exclusively anomeric pairs of the N-acetyl-1-deoxy-1-nitromannosamine derivatives (Scheme 19).

Starting with such a N-acetyl-1-deoxy-1-nitromannosamine derivative and a tert-butyl acrylate the synthesis of Neu5Ac and 4-epi-Neu5Ac was elaborated. Michael addition of 1-nitromannosamine derivatives to tert-butyl 2-(bromomethyl)prop-2-enoate (54) and subsequent hydrolytic removal of the nitro group gave chain-elongated nonulosonates (Scheme 22), which

upon stereoselective reduction with $\text{NaBH}_4/\text{AcOH}$ in dioxane-water resulted favorably in the D-glycero-D-galacto configurated triols 147 and 149, respectively. These triols were converted into Neu5Ac. Reduction of the nonulosonates with NaBH_4 in the absence of AcOH or in EtOH gave favorably triols with the D-glycero-D-talo configuration forming N-acetyl-4-epineuraminic acid finally.

Exploiting our synthesis of Neu5Ac and 4-epi-Neu5Ac we prepared the N-acetyl-4-deoxyneuraminic acid (162). An epimeric mixture of the triols 147/148 was acetylated, ozonized, hydrogenated and deprotected to give 4-deoxy-Neu5Ac in a good yield (Scheme 23 and 24).

To test the N-acetyl-4-deoxyneuraminic acid (162) in a glycosidase-assay we synthesized the 4-methylumbelliferyl α -D-glycoside 181 of N-acetyl-4-deoxyneuraminic acid by a Königs-Knorr glycosidation, applying a similar procedure as described for the corresponding glycoside of Neu5Ac (Scheme 25). The α -D-glycoside 181 is not hydrolized at significant rates by Vibrio cholerae and Arthrobacter ureafaciens sialidase, respectively. Neither the free N-acetyl-4-deoxyneuraminic acid (162), nor the α -D-glycoside 181 inhibit the activity of these sialidases.

By analogy with several piperidine derivatives (186-193), which are glycosidase inhibitors, 6-amino-6-deoxy-sialic acids may be sialidase inhibitors. Mitsunobu reaction of the 1-C-nitroglycal 123 (PPh_3 , HCOOH , DEAD) gave a nitro olefin with inversion of configuration at C(3). Treatment of this nitro olefin with aq. NH_3 and subsequent protection of the amino function gave an imine (212; Scheme 30), which after transformation into a triflate and displacement of the triflic group by LiN_3 , followed by deprotection and acetylation of the C(2) amino function resulted in a 2-acetamido-3-azido-1-deoxy-1-nitro-D-mannose (215). Similarly to the synthesis of Neu5Ac, the azide 215 was chain-elongated, hydrolized and the resulting nonulosonate was reduced giving the diols 220 and 221, respectively (Scheme 31). The diol 220 was transformed into the sialic acids 227 and 228, whereupon the diol 221 was converted into the sialic acid 230. In both cases standard reactions (ozonolysis, transfer hydrogenation, hydrogenolysis and deprotection) were used. The configuration of the sialic acids

was assigned by spectroscopic methods and by comparison of the data with those of the corresponding Neu5Ac derivatives.

9. ZUSAMMENFASSUNG

Die Grundlagen für die Synthese von Sialinsäuren ausgehend von 1-Desoxy-1-nitroaldosen wurden ausgearbeitet. Die Synthesen von Neu5Ac, 4-epi-Neu5Ac, 4-desoxy-Neu5Ac, des 4-Methylumbelliferyl- α -D-glycosids von 4-desoxy-Neu5Ac und die Herstellung von 6-Amino-6-desoxysialinsäuren werden beschrieben.

Die radikalische Denitrierung der Pyranosederivate 64, 65, 70 und 71 mit Bu_3SnH ergab diastereoselektiv und in guten Ausbeuten die 'C-Glycoside' 66, bzw. 72 (Schema 11). Zum Vergleich wurden die Cyclohexylderivate 76, 77, 80 und 81 unter gleichen Bedingungen denitriert. Diese lieferten 4:1 Gemische der Verbindungen 78/79, bzw. 82/83 (Schema 12). Das Verhalten bei der reduktiven Denitrierung von Furanosederivaten war unterschiedlich. Während sich die von D-Mannose abgeleiteten Nitroäther 86 und 88 diastereoselektiv in die Produkte 87, bzw. 89 umsetzen liessen (Schema 13), reagierten die D-Ribosederivate 91 und 92 zu 1:1 Gemischen der Diastereomeren 93 und 94 (Schema 14). Das unterschiedliche Verhalten der D-Mannose- bzw. D-Ribosederivate wurde mit Hilfe von stereoelektronischen und sterischen Effekten des Ringsauerstoffatoms erklärt.

Analog zu einem bekannten Verfahren für die Herstellung von geschützten 1-Desoxy-1-nitroaldosen, wurde die ungeschützte 1-Desoxy-1-nitro-D-mannopyranose 115 hergestellt. Diese wurde mit 2-Methoxypropen in die 4,6-O-isopropyliden geschützten Mannosederivate 116/117 umgesetzt. Ein Gemisch dieser Anomeren ergab nach Acetylierung und Basenbehandlung das 1-C-Nitroglycal 118 (Schema 16). In gleicher Weise stellte man die verschiedenen geschützten Nitroolefine 121, 123, 127 und 129 her (Schema 18). Die β -Addition von NH_3 an 1-C-Nitroglycale war gefolgt von einer O $\rightarrow$ N-Acetylwanderung und lieferte ausschliesslich die Anomerenpaare von N-Acetyl-1-desoxy-1-nitro-D-mannose (Schema 19).

Die Kettenverlängerung der so hergestellten Mannosaminderivate um eine C-3-Einheit war die Basis für die Synthese verschiedener Sialinsäuren. Michael-Addition der N-Acetyl-1-desoxy-1-nitromannosen an den 2-(Bromomethyl)acrylsäure-Ester 54 und nachfolgende hydrolytische Entfer-

nung der Nitrogruppe lieferte Nonulosonsäure-Ester (Schema 22). Stereoselektive Reduktion dieser Nonulosonsäurederivate mit $\text{NaBH}_4/\text{AcOH}$ in Dioxan/ H_2O ergab bevorzugt die D-glycero-D-galacto-konfigurierten Triole. Durch Standardreaktionen (Ozonolyse, Entschützen) wurden diese Triole in N-Acetylneuraminsäure umgewandelt. Die Reduktion der Nonulosonsäure-Ester mit NaBH_4 in Abwesenheit von AcOH lieferte bevorzugt D-glycero-D-talo-konfigurierte Triole, die nach Ozonolyse und Entfernung der Schutzgruppen N-Acetyl-4-epineuraminsäure ergaben.

Analog wurde auch N-Acetyl-4-desoxyneuraminsäure hergestellt. Ein Gemisch der epimeren Alkohole 147/148 (Zwischenprodukte bei der Synthese von Neu5Ac) wurden acetyliert und nachfolgend mit Ozon behandelt. Bei der Aufarbeitung des Reaktionsgemisches erfolgte eine spontane β -Eliminierung von AcOH unter Bildung des β,γ -ungesättigten α -Ketoesters 165 (Schema 23). Nach Hydrierung der Doppelbindung und Entschützen des Folgeproduktes erhielt man in guten Ausbeuten N-Acetyl-4-desoxy-neuraminsäure (162, Schema 24).

α -D-Glycoside der N-Acetylneuraminsäure werden durch Sialidasen gespalten. Um das Verhalten eines Glycosids der N-Acetyl-4-desoxyneuraminsäure gegenüber Sialidasen zu testen, synthetisierten wir das 4-Methylumbelliferyl- α -D-glycosid 181 von 4-desoxy-Neu5Ac (Schema 25). Unter Königs-Knorr-Bedingungen erhielt man das Glycosid 181 in guten Ausbeuten. Das α -D-Glycosid 181 wird durch *Vibrio cholerae*- und *Arthrobacter ureafaciens*-Sialidase nur sehr langsam gespalten (1,4% der Hydrolysegeschwindigkeit von MU-Neu5Ac). Weder das Glycosid 181 noch die freie N-Acetyl-4-desoxyneuraminsäure (162) inhibieren die Sialidase-Aktivität.

Mehrere Piperidinderivate z.B. 186-193 sind Glycosidaseinhibitoren. Analog zu diesen Piperidinen könnten 6-Amino-6-desoxysialinsäuren Sialidaseinhibitoren sein. Das Nitroolefin 123 wurde durch Mitsunobu-Reaktion (Ph_3P , HCOOH , DEAD) in ein Nitroolefin 207 mit inverser Konfiguration an C(3) umgewandelt (Schema 29). Addition von NH_3 an das Olefin und nachfolgendes Schützen der Aminofunktion führte zum Imin 212 (Schema 30). Die Hydroxygruppe an C(3) überführte man in eine Trifluoromethansulfonylgruppe und substituierte diese durch LiN_3 . Die Aminofunktion an C(2)

wurde entschützt und N-acetyliert, was die 2-Acetamido-3-azido-1-desoxy-1-nitro-D-mannose 215 lieferte. Verbindung 215 wurde (analog wie oben beschrieben) kettenverlängert, die Nitrogruppe hydrolytisch entfernt und die erhaltene Nonulosonsäure zu den Diolen 220 und 221 reduziert (Schema 31). Das Diol 220 wandelte man um in die 6-Amino-6-desoxysialinsäuren 227 und 228. Das Diol 221 führte nach weiteren Umsetzungen zur Sialinsäure 230. In beiden Fällen kamen Standardreaktionen (Ozonolyse, Transferhydrogenierung, Hydrierung) zur Anwendung. Die Konfiguration der erhaltenen Sialinsäuren wurde mit Hilfe spektroskopischer Methoden und durch Datenvergleich mit den entsprechenden N-Acetylneuraminsäurederivaten abgeleitet.

10. REFERENCES

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